Abbreviated Title: TMZ in breast ca brain mets
NIH Protocol: 17-C-0115
Version Date: 06/06/2022
NCT Number: NCT03190967

Title: Phase I/II Study of T-DM1 alone versus T-DM1 and Metronomic Temozolomide in Secondary Prevention of HER2-Positive Breast Cancer Brain Metastases Following Stereotactic Radiosurgery

NCI Principal Investigator: Stanley Lipkowitz, MD, PhD, WMB, CCR, NCI
Women's Malignancies Branch
National Cancer Institute
Building 10, Rm 4B54
9000 Rockville Pike
Bethesda, MD 20892
Phone: 240-760-6129
E-mail: lipkowis@navmed.nci.nih.gov

Investigational Agents: None

Commercial Agents: T-DM1, Temzolomide

PRÉCIS

Background:

- Breast cancer is the most common cancer in women. In the HER2+ subtype, brain metastases can occur in up to 25-40% of patients.
- The standard therapy for brain metastases continues to be surgery or stereotactic radiosurgery (SRS) and/or whole brain radiation therapy (WBRT).
- Currently, independently of localized or systemic treatment modality, once brain metastases are established, options for treatment are limited, and the disease almost invariably progresses, limiting not only survival but also quality of life in most patients.
- Preclinical literature suggests the hypothesis that preventing the formation of a metastasis by a drug may be more efficacious than attempting to shrink an established lesion.
- Our group has shown *in vitro* and *in vivo* in animal models injected with a brain tropic MGMT+ cell line, that even in very low doses temozolomide (TMZ) administered in a prophylactic, metronomic fashion can significantly prevent development of brain metastases.
- We propose a secondary-prevention clinical trial with oral TMZ given to HER2+ breast cancer patients with brain metastases after recent local treatment (SRS or surgical resection) in combination with the anti-HER2 agent T-DM1 for systemic control of disease.

Objectives:

- Phase I (run in): to identify the maximum tolerated dose (MTD) of TMZ when used in combination with T-DM1.
- Phase II: to determine if the combination regimen of T-DM1 and temozolomide improves the freedom from distant new brain metastases following stereotactic radiosurgery or surgical resection in HER2-positive breast cancer brain metastases, as compared to T-DM1 alone, guided by one-year results as an important benchmark for measuring improvement.

Eligibility:

<u>Phase I</u>:

- Histologically confirmed HER2+ breast cancer.
- ECOG performance status 0-2 and adequate organ and marrow function.
- Brain metastases, treated within 12 weeks of study entry with SRS, resection or WBRT.
- Patients with leptomeningeal metastatic disease are ineligible.
- Patients that are unable to complete a brain MRI with contrast are ineligible.
- Patients with breast tissue expanders must have those removed before enrollment.
- HBV, HCV or HIV-positive patients are ineligible.
- Patient with impaired cardiac function or clinically significant cardiac disease are ineligible.

- Corticosteroids will be allowed at enrollment and during the first month of treatment with T-DM1 after SRS, up to a dose of no more than 10mg of dexamethasone daily or equivalent. Patients that need to continue corticosteroids after the initial month will not be allowed to increase the dose after that period, and will be taken off protocol.

Phase II:

- Histologically confirmed HER2+ breast cancer.
- ECOG performance status 0-2 and adequate organ and marrow function.
- 1-10 brain metastases, by contrast MRI, treated within 12 weeks of study entry with SRS and/or resection.
- Patients with leptomeningeal metastatic disease are ineligible.
- Patients with history of WBRT are ineligible.
- Patients that are unable to complete a brain MRI with contrast are ineligible.
- Patients with breast tissue expanders must have those removed before enrollment.
- HBV, HCV or HIV-positive patients are ineligible.
- Patient with impaired cardiac function or clinically significant cardiac disease are ineligible.
- Corticosteroids will be allowed at enrollment and during the first month of treatment with T-DM1 after SRS, up to a dose of no more than 10mg of dexamethasone daily or equivalent. Patients that need to continue corticosteroids after the initial month will not be allowed to increase the dose after that period, and will be taken off protocol.

Design:

- This is a Phase I/II open label study that will evaluate the potential benefit of TMZ in prevention of new brain metastases in patients with limited brain metastases from HER2+ breast cancer, previously treated with SRS or surgical resection of brain metastases.
- All patients will receive the standard second-line therapy for HER2+ metastatic breast cancer: T-DM1. During phase II patients will be randomized between T-DM1 plus TMZ versus T-DM1 alone.
- Phase I run in: T-DM1 3.6 mg/kg IV every 21 days plus TMZ 30, 40 or 50 mg/m2 daily.
- Phase II: T-DM1 3.6 mg/kg versus T-DM1 3.6mg/kg plus TMZ at recommended phase 2 dose (RP2D).
- Phase I will follow a standard 3+3 design. Thus, with 3 dose levels, up to 18 patients may be included in the initial safety evaluation.
- In the phase II portion of the trial, a total of 49 evaluable subjects per arm (98 total) will need to be randomized over a 3-year period and followed for an additional 2 years from the date of entry of the last patient, with occurrence of 79 total relapses in both arms combined, in order to have 80% power to compare the curves.

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives:

Phase I (run in):

- To identify the maximum tolerated dose (MTD) of temozolomide when used in combination with T-DM1.

Phase II:

- To determine if the combination regimen of T-DM1 and temozolomide improves the freedom from distant new brain metastases following stereotactic radiosurgery or surgical resection in HER2-positive breast cancer brain metastases as compared to T-DM1 alone guided by one-year results as an important benchmark for measuring improvement.

1.1.2 Secondary Objective(s):

- To evaluate the safety and tolerability of the combination of T-DM1 and temozolomide in HER2-positive breast cancer patients, previously treated for limited brain metastases.
- To assess time to whole brain radiation, and overall patient survival when treated with the combination regimen, compared to T-DM1 alone (control arm).

1.1.3 Exploratory Objective(s):

- To assess tumor genomic and neuroinflammatory biomarkers in the CSF and serum; the effects of treatment in those biomarkers; and the ability to predict relapses using those biomarkers; as described in the correlative studies background section (Section **1.3**).To evaluate MGMT methylation status in primary tumor, CSF and serum; to compare it between patients that benefit and those that don't benefit from treatment with temozolomide.

- To assess neurotoxicity and neurocognitive effects in patients treated with the combination regimen compared to the control arm.
- To assess between arm differences in symptom burden as measured by the M.D. Anderson Symptom Inventory-Brain Module (MDASI-BT). Specifically, it is hypothesized that the following subscales on the MDASI-BT (cognitive dysfunction, focal neurologic deficits and interference) will be higher in those receiving temozolomide alone due to differences in time to disease progression.
- To assess if baseline MDASI-BT subscale scores (overall symptom burden, overall interference, cognitive dysfunction and focal neurologic deficits) along with the specific items of fatigue and change in appetite will be prognostic for time to distant metastases and for OS.
- To assess if change scores over time in MDASI-BT subscale (overall symptom burden, overall interference, cognitive dysfunction, and focal neurologic deficits) along specific items (difficulty concentrating, fatigue, nausea, and headaches) were prognostic for OS, time to distant metastases, and neurocognitive decline.

1.2 BACKGROUND AND RATIONALE

1.2.1 Brain metastasis in HER2 positive breast cancer

Breast cancer is the most common cancer in women [1], and the second most common cause of brain metastases in the United States [2]. The incidence of brain metastases varies amongst the different molecular subtypes of breast cancer, with high incidence in the HER2-positive and triple-negative breast cancers [3,4]. In the HER2-positive group, the diagnosis of brain metastases became even more frequent – up to 25-40% [5-8] - after widespread treatment with trastuzumab, a monoclonal antibody that improved survival and control of systemic disease but has low central nervous system penetrance [9,10]. New agents targeting HER2 have been evaluated since the introduction of trastuzumab, and, though several are now proven effective for systemic control of the disease, a major effect in decreasing brain metastases incidence in this group is still lacking.

1.2.2 Treatment of brain metastases

1.2.2.1 Local therapy

Radiation therapy modalities are currently the main options for treatment of brain metastases. In patients with limited number of brain metastases (one to five), current treatments include surgical resection, stereotactic radiosurgery (SRS), and/or whole-brain radiotherapy (WBRT) [11]. Most prospective trials evaluating local treatment of brain metastases included mainly NSCLC patients and only a small proportion of breast cancer patients [12-14]. Addition of WBRT to initial surgery or SRS has repeatedly demonstrated a decrease in intracranial disease recurrence but no difference in the overall survival of 7 to 10 months. However, WBRT has been reported to worsen quality of life and neurocognitive function, especially in patients with prolonged survival [15-17]. In those cases, neurocognitive decline is progressive and untreatable. WBRT alone is the main indicated treatment for patients with higher numbers of brain metastatic lesions.

1.2.2.2 Systemic therapy

Systemic therapy for brain metastases, overall, has shown less efficacy than in systemic, non-CNS locations. Multiple clinical trials have documented few or no responses using agents with known activity in the metastatic setting [18-23]. The reasons for this may be heterogeneous, but likely involve the blood-brain barrier (BBB), which prevents most substances from exiting the bloodstream and entering the brain parenchyma. Once a metastasis forms the BBB is transformed into a blood-tumor barrier (BTB). In general, the BTB permits greater drug penetration than the BBB, but drug levels are ~a log less than those reached in systemic metastases and therefore ineffective [24].

In the HER2 positive breast cancer patients, HER2 targeted agents beyond trastuzumab have been also evaluated for their potential therapeutic effect in brain metastases. Lapatinib is a small molecule tyrosine-kinase inhibitor of epidermal growth factor receptor (EGFR) and human epidermal growth factor type 2 (HER2), able to cross the BTB to a greater extent than paclitaxel [24,25]. A presurgical study documented heterogeneous levels of lapatinib in brain metastases from patients dosed pre-operatively for medically needed craniotomies[26] .

As a single agent, lapatinib showed a few responses and a small non-significant diminution in size of brain metastatic lesions [27,28]. The combination lapatinib plus capecitabine produced objective responses in approximately 30% of patients in phase II trials evaluating the combination treatment in patients previously treated with WBRT [28,29]. The LANDSCAPE trial evaluated the combination in a single arm phase II design as first-line treatment for low-volume brain metastases with objective CNS responses of 65.9%, median time to CNS progression of 5.5 months, and median time to WBRT of 8.5 months [30]. More recently, the MA.31 trial randomized 652 patients with HER2-positive breast cancer to treatment with lapatinib plus taxane or trastuzumab plus taxane as first line treatment of metastatic disease [31]. The primary endpoint was PFS. The trastuzumab combination was superior to the lapatinib combination, 9.0 months and 11.3 months, respectively (HR 1.37, p=0.001). The incidence of brain metastases as first site of progression was 28% for trastuzumab and 20% for lapatinib, with no difference in time to progression between the arms. In a brain metastasis prevention setting, a phase III trial of lapatinib plus capecitabine versus capecitabine alone in HER2-positive advanced breast cancer previously treated with an anthracycline, taxane, and trastuzumab was conducted. 4 (2%) patients developed symptomatic brain metastasis as an initial site of progression in the combination therapy arm compared to 13 (6%) patients in the monotherapy group (p=0.045) [32].

Pertuzumab is a monoclonal antibody that targets HER2 extracellular dimerization domain and acts in synergy with trastuzumab. It was demonstrated to prolong survival when used with trastuzumab and docetaxel as first line treatment for metastatic HER2-positive breast cancer, compared to trastuzumab, docetaxel and placebo [33]. An exploratory analysis of the trial evaluated the incidence of brain metastases as first site of disease progression, and found it to be similar in the pertuzumab arm and the placebo arm (13.7% and 12.6%)[34].

Trastuzumab emtansine (T-DM1) is a newer anti-HER2 therapeutic agent, with case reports describing activity in CNS metastases [35,36]. T-DM1 is an antibody–drug conjugate containing emtansine (DM1), a microtubule-inhibitory agent, linked to trastuzumab. Phase III trials have shown activity of T-DM1 in HER2-positive metastatic disease after previous lines of treatment including trastuzumab and lapatinib, with an increase in PFS and OS [37,38]. T-DM1 is currently the standard therapy for HER2-positive breast cancer patients with recurrence or progression of disease after treatment with trastuzumab. The EMILIA trial randomized advanced HER2-positive

breast cancer patients previously treated with trastuzumab and taxanes, to receive either T-DM1 or lapatinib plus capecitabine [38]. Final results showed better PFS (9.6 months with T-DM1 *versus* 6.4 months with lapatinib plus capecitabine - hazard ratio for progression or death from any cause, 0.65; 95% confidence interval [CI], 0.55 to 0.77; P<0.001), and OS (30.9 months *versus* 25.1 months; hazard ratio for death from any cause, 0.68; 95% CI, 0.55 to 0.85; P<0.001) for T-DM1. In a retrospective exploratory analysis of the EMILIA trial, the rate of CNS progression was similar between the two arms, with CNS metastases as the first site of relapse in 2% of T-DM1 treated patients and in 0.7% of lapatinib plus capecitabine treated patients, and progression of CNS disease known at baseline in 22.2% and 16%, respectively. However, in patients with treated asymptomatic CNS metastases at baseline, T-DM1 was associated with improved survival when compared to lapatinib plus capecitabine (26.8 versus 12.9 months, HR 0.38 p=0.008) [39]. One report raised concerns about possible inflammatory interaction between T-DM1 and SRS-treated lesions with clinically symptomatic edema, not largely seen outside this report but it requires close monitoring [40].

Independently of localized or systemic treatment modality, once brain metastases are established, our options for treatment are currently limited and the disease almost invariably progresses, limiting not only survival but also quality of life in most patients.

1.2.3 Prevention of brain metastases

Preventing the development of brain metastases in high-risk breast cancer patients is a different way to tackle the problem clinically. Primary prevention refers to the initial development of a brain metastasis; secondary prevention refers to the development of additional brain metastases in patients with limited metastases treated with local therapy.

The preclinical literature suggests the hypothesis that preventing the formation of a metastasis by a drug may be more efficacious than attempting to shrink an established lesion. Multiple mouse models of brain metastasis of breast cancer have been developed. Many approved drugs and candidates have been tested in a prevention mode, where they were delivered early and continuously after tumor cell injection, or in a therapeutic setting, where brain lesions were permitted to form and then treatment began. As reviewed[41], several drugs have shown statistically significant efficacy in preventing the formation of brain metastases. None have shown therapeutic efficacy, in keeping with the clinical literature.

The use of prophylactic radiation therapy, as used in small-cell lung cancer, is one approach to prevent brain metastases in high risk patients. At least two trials, one in Europe (NCT00639366) and other in Canada (NCT00916877), recruited high-risk breast cancer patients to evaluate prophylactic radiation and prevention of brain metastases and results are still maturing. The increasing evidence of neurocognitive decline related to WBRT and the lack of initial reports showing major sign of positive results are discouraging though [42]. There is increasing evidence of at least transient declines in memory, executive function, and processing speed in the first few months after WBRT in up to 40-60% patients. Based on the literature, additional progressive neurocognitive complications may be expected with time.

In an attempt to evaluate prophylactic treatment against brain metastases as first site of relapse, the phase III CEREBEL trial assessed 501 women that were randomized to lapatinib plus capecitabine *versus* trastuzumab plus capecitabine [43]. The incidence of brain metastases was overall low, 3% and 5% respectively, with no significant difference between the two arms.

Considering the different subtypes of breast cancer, a retrospective analysis of 154 patients (56% HER2-positive) treated with SRS, evaluated their outcome in terms of new brain metastases, overall survival and death ultimately caused by progression in brain [44]. Median overall survival was 16.7 months for HER2-positive patients and 8.4 months for HER2-negative patients ($p<0.001$). Brain metastasis progression was the cause of death in 46% of HER2-positive and 31% of HER2-negative patients ($p=0.066$). Freedom from distant brain failure at 1 year was 58% in HER2-positive as opposed to 40% in HER2-negative patients ($p=0.034$), however, the majority of those patients had also received WBRT in the past. Such analysis reinforces the findings that, once brain metastases are found, HER2-positive patients may have a better overall survival, but they are more prone to develop and to die from brain metastasis progression. Most interestingly, since brain metastatic disease frequently recurs after initial SRS, a secondary prevention strategy could be helpful at this point.

Temozolomide in brain metastases

Temozolomide (TMZ) is a cytotoxic alkylating agent with almost 100% oral bioavailability, known to penetrate the BBB and currently FDA approved for treatment of glioblastoma multiforme and recurrent anaplastic astrocytoma. Although not proven effective as single agent to treat established metastatic breast cancer [45], TMZ has been extensively evaluated in the treatment of established brain metastases in combination with other agents and/or radiation. therapy, showing mixed responses and good tolerability [46-56].

Table 1. Prevention of experimental brain metastases by TMZ over a wide dose range.

Experiment:	Dose (mg/kg)	Median number Metastases Per Brain Section:	
		Large:	Micro:
1	0	2.0	63.3
	50	0	0
2	0	2.6	86.4
	50	0	0
	25	0	0
3	0	6.5	143.3
	25	0	0
	10	0	0
	5	0	0
4	0	2.3	101.1
	1	0.9	40.1
	0.5	1.2	58.0

Mice were injected with brain tropic MDAMB231-BR cells and treated from day 3 post-injection with the indicated dose of TMZ. At necropsy large (>300 um) and micrometastases were quantified in step sections through one brain hemisphere. Experiments 1-3, $p< 0.01$. Results of four experiments are shown to demonstrate the efficacy of TMZ dose.

Our group has shown in models developed with brain tropic HER2–positive JIMT-1-BR3 (*in vitro*) and triple-negative 231-BR-EGFP (*in vitro* and *in vivo* in animal models) sublines, that even in very low doses TMZ administered in a prophylactic fashion can prevent development of brain metastases (**Table 1**) [57]. The brain tropic tumor cells were injected into left cardiac ventricle and on day 3 the mice were randomized to vehicle *versus* TMZ given by oral gavage for 5 days every week. At necropsy, brain metastases were quantified in step sections through one hemisphere, and

doses of TMZ from 5 to 50mg/kg significantly prevented brain metastases formation. TMZ was ineffective in the treatment (shrinking) of established brain metastases. When mice injected with brain-tropic breast cancer cells were treated with a course of TMZ and then allowed to live untreated, TMZ extended survival, with early treatment better than later (**Figure 1**). The ability of TMZ to prolong life more effectively when given earlier is consistent with its ability to prevent metastases but inability to treat established metastases.

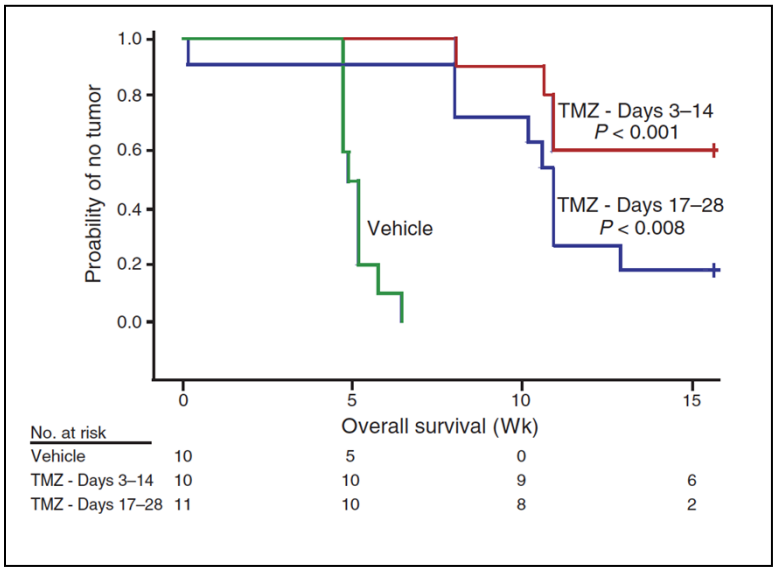


Figure 1. MDA-MB231-BR cells were injected into nude mice; Mice were treated with vehicle or TMZ on the days post-injection indicated. Mice were allowed to age without further treatment. They were sacrificed upon signs of progression and a Kaplan-Meier graph of survival is shown.

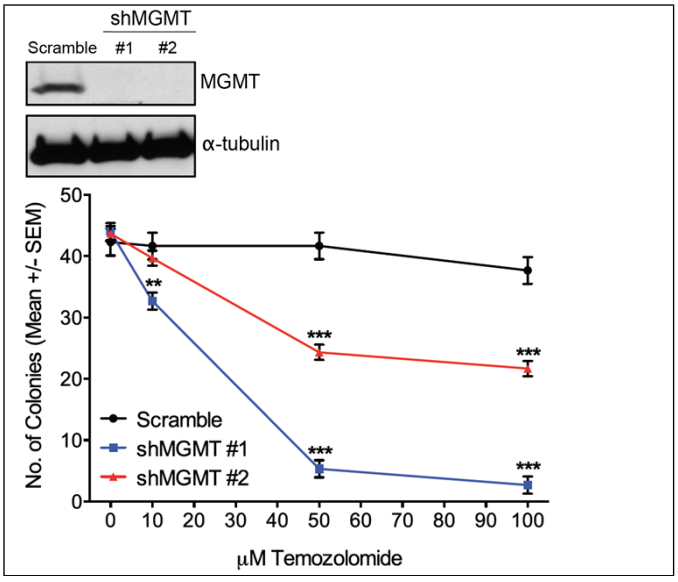


Figure 2. Temozolomide is ineffective at preventing colonization of MGMT+ Jimt-1-BR3 cells. Top panel: Jimt-1-BR3 cells were transduced with two different MGMT-targeting shRNAs, along with a scrambled shRNA control. Reduced MGMT level in Jimt-1-BR3 cells transduced with targeted shRNAs was confirmed by western blot. Expression level of α-tubulin served as loading control. Lower panel: Altered sensitivity to temozolomide following MGMT repression was assessed in clonogenic survival assays. Jimt-1-BR3 cells were seeded at single cell density in triplicate, allowed to attach for 24h, then treated with either vehicle or

**temozolomide at 10, 50 or 100 µM final concentrations. Colonies were fixed, stained with crystal violet and counted at 14 days posttreatment. Colony counts are shown as mean +/- SEM; **P=.008, *****P

O^6-Methylguanine-DNA methyltransferase (MGMT) is critical for repairing pro-mutagenic DNA bases and known to be contributory to tumor resistance to alkylating agents [58,59]. Tumor response to TMZ has been shown to depend on low MGMT expression both in glioblastomas and in our preclinical breast cancer models as well (**Figure 2**) [57,60]. The correlation between primary breast tumor expression of MGMT and metastatic tissue expression of MGMT has not been largely investigated. Our group also evaluated 62 primary breast cancer tumor samples and their patient-matched brain metastases (**Figure 3**). Primary breast tumor MGMT expression was not a reliable predictor of brain MGMT status, with 40% discordance between sites. In our sample, 60% of patients with breast cancer brain metastases showed low MGMT expression (**Table 2**), indicating that these patients are potentially susceptible to TMZ prevention of brain metastasis. A strong trend was observed for an association of HER2 overexpression and MGMT negativity in the HER2 positive brain metastases (P = 0.089), suggesting the eligibility of this subset of patients for potential clinical trials (**Table 3**) [57].

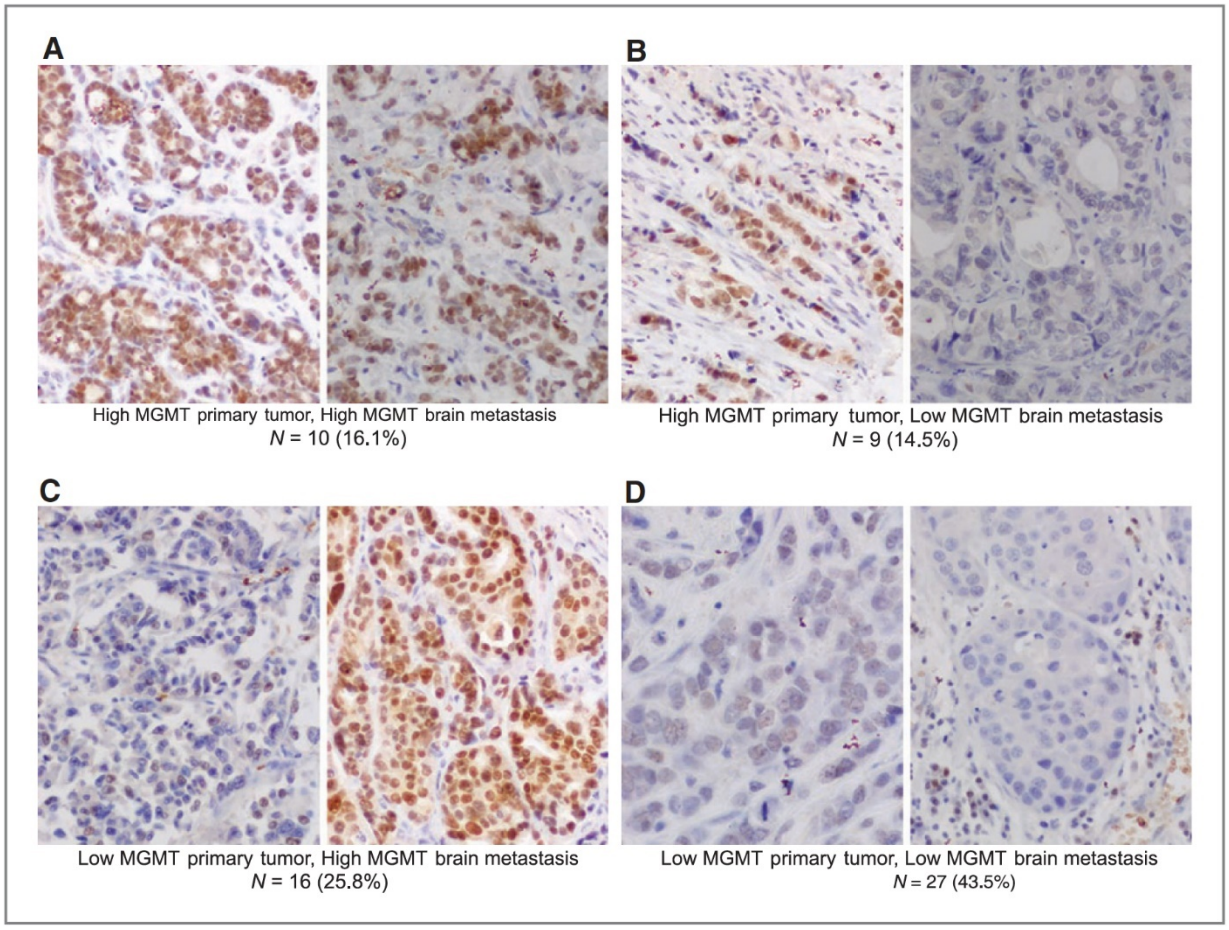


Figure 3. MGMT expression in matched sets of human breast primary tumors and resected brain metastases. A- D, sixty-two patient-matched sets were collected from tumor banks in Poland and Germany. TMAs of the specimens were stained for MGMT and evaluated for the percentage of positively staining tumor cells (nuclear staining only), dichotomized at 5%. The number and percentage of specimens in each category is given below each representative photomicrograph

Table 2. Cross-tabulation of MGMT IHC staining in matched primary breast tumors and resected brain metastases using two categories[a].

Primary tumor	Brain metastases		Total
	<5% MGMT+	>5% MGMT+	
<5% MGMT +	27 (44%)	16 (26%)	43 (70%)
>5% MGMT +	9 (14%)	10 (16%)	19 (30%)
Total	36 (58%)	26 (42%)	62 (100%)

[a] The percentage of MGMT+ cells was scored by a pathologist, and grouped into four categories as listed. P=0.036 by Jonckheere-Terpstra trend test

TMZ has been administered in at least two doses and schedules. In its activity repairing O^6-methylguanine lesions, MGMT transfers the alkyl group from guanine to a cysteine moiety in its active site, thereby repairing the DNA. MGMT is irreversibly inactivated in this process and new MGMT protein synthesis is needed to restore MGMT activity [61,62]. In an attempt to overcome MGMT activity, trials evaluating the metronomic use of temozolomide were developed mainly for treatment of melanoma and brain tumors [63,64]. MGMT enzyme activity was assayed in peripheral blood monocytes (PBMCs) collected at various time points during treatment and shown to be progressive and cumulatively decreased [65,66]. More recently, at least three phase II clinical trials evaluated the continuous daily use of temozolomide (50mg/m2) in heavily pretreated brain tumors and demonstrated activity and safety of the regimen, even in non-methylated MGMT tumors [54,67,68].

To our knowledge, the only published trial evaluating the combined use of temozolomide with an anti-HER2 agent in breast cancer brain metastases was the LAPTEM trial [50], combining temozolomide with lapatinib for treatment of established brain metastases, not prevention. The regimen showed a favorable toxicity profile, with main side effects being fatigue, diarrhea and constipation. Temozolomide was used in three dose levels: 100, 150 and 200 mg/m2/day, days 1–5 with cycles of 28 days; lapatinib was given orally, once a day also at three dose levels: 1000, 1250, 1500 mg/day. The MTD was not reached, per protocol. No cardiac toxicity events were observed. Of the 15 evaluable patients, 10 achieved stabilization of disease, with estimated median survival time of 10.94 months (95% CI: 1.09–20.79), and median progression-free survival time of 2.60 months [95% CI: 1.82–3.37].

We propose a secondary-prevention clinical trial with oral temozolomide for HER2-positive breast cancer patients with 1 to 3 brain metastases after recent local treatment (SRS or surgical resection), with the goal to decrease incidence of new metastatic lesions in the brain at 1 year. Temozolomide will be combined to the anti-HER2 agent T-DM1 for systemic control of disease.

Table 3Associations of primary tumor and brain metastasis MGMT expression with patient characteristics

Variable	Number with characteristic/total (%)		P
	MGMT-negative (≤5% positive tumor cells)	MGMT-positive (>5% positive tumor cells)	
Brain metastases (n = 36 and 26, respectively)			
Receptor status			
ER-positive	16/27 (59%)	13/21 (62%)	1.0
PR-positive	9/26 (35%)	10/21 (48%)	0.39
HER2-positive	16/27 (59%)	7/21 (33%)	0.089

1.3 CORRELATIVES BACKGROUND AND RATIONALE

Studies will address aspects of TMZ resistance, molecular alterations in brain metastatic tumor cells, the neuro-inflammatory response, and markers of cognition.

1.3.1 Cell free DNA (cfDNA) and primary tumor DNA sequencing

Few molecular tools exist to analyze brain metastasis progression other than MRI scans and neurocognitive testing. Recent work from Harvard demonstrated that brain metastases show unique mutational patterns as compared to primary tumors [69]. An example is shown on **Figure 4.** These data were based on craniotomy samples, which will generally be unavailable in the current clinical study. Detecting DNA alterations in brain metastasis from patients' systemic samples will likely be compromised by the contributions of systemic disease. We propose sequencing of cell free tumor DNA in the CSF as an alternative endpoint.

Recently several publications have reported the sequencing of cell free DNA (cfDNA) in CSF from patients with brain cancers and leptomeningeal disease from melanoma [70,71]. The primary tumor tissue was subjected to DNA sequencing to identify specific alterations from a preselected targeted panel. In one report four CSF samples were analyzed by whole exome sequencing without prior knowledge of specific mutations; two of the four revealed mutant allele frequencies >10% that could be tracked [70]. The presence or absence of cfDNA in CSF, or its levels and alterations was a correlate of clinical course and more informative than serum cfDNA[71]. Based on these data it is hypothesized that: (a) patients will have mutations that can be identified in the CSF in the secondary brain metastasis prevention trial; (b) alterations in the pattern or prevalence of mutations will correlate with clinical outcome in the secondary brain metastasis prevention trial and (c) that alterations in the pattern or prevalence of mutations or cfDNA levels in CSF will predict relapse before MRI imaging. In addition, the data will provide a landscape of the genetic mutations from cfDNA in CSF for brain metastasis of breast cancer, and a portrait of its evolution through progression. The CSF cfDNA sequence data will be compared to that of the primary tumor where available and serum cfDNA. Comparison with the primary tumor may provide a portrait of mutations that are shared between the primary tumor and the brain metastasis as evidenced in cfDNA from CSF. Comparison with serum may identify events distinct between systemic and CNS progression.

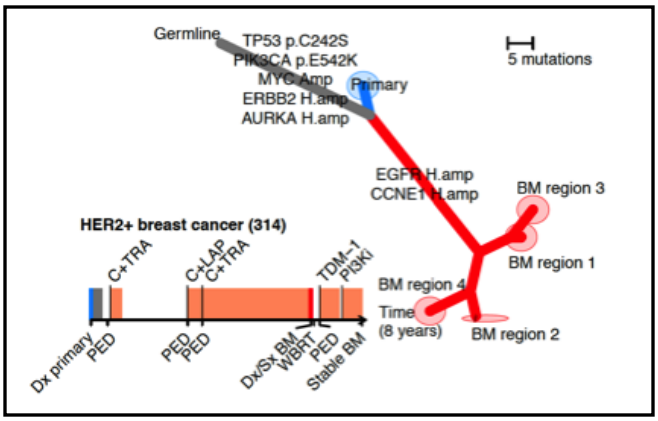


Figure 4: Phylogenetic tree for a patient that developed a brain metastasis of HER2-positive breast cancer, as compared to the matched primary tumor. The phylogenetic tree is presented on the top, with the patient treatment history below. Primary tumor data is blue. All regions of the brain metastases (red) shared amplifications in *EGFR* and *CCNE1* that were absent in the primary tumor. Heterogeneity was also observed in sequences from four different regions of the resected brain metastasis. In gray are mutational events shared by all samples. The timeline depicts the sequence of diagnosis, treatment, and tissue sampling, with chemotherapy treatment intervals denoted by grey rectangles, and treatment with specified targeted agents denoted by orange rectangles. Data developed by Drs. Brastianos and Carter.

This approach has been pioneered by Drs. Carter and Brastianos and is potentially groundbreaking. Drs. Brastianos and Carter have already subjected nearly 100 matched brain metastases, primary tumors and normal tissues to whole exome sequencing and demonstrated that in 53% of cases, oncogenic drivers were detected in the brain metastases that were not present in the clinically sampled primary tumor[69]. A case of a resected HER2+ brain metastasis is presented in **Figure 4**, showing that the brain metastasis was genetically divergent from the primary tumor biopsy and that multiple regions of the brain metastasis were relatively genetically homogeneous. Now they have developed techniques to analyze cfDNA in CSF[69,72-74].

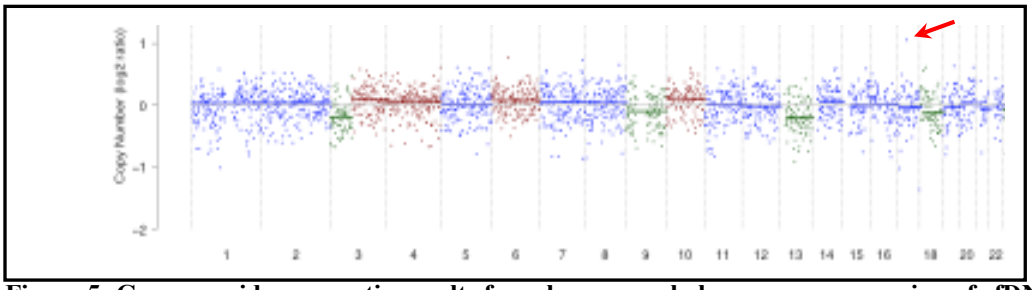


Figure 5: Genome-wide copy ratio results from low-pass whole-genome sequencing of cfDNA derived from CSF of a patient with metastatic HER2+ breast cancer. Analysis of chromosomal aneuploidies in the sample indicates a tumor purity of 14% in the cfDNA. Note that the *ERBB2* amplicon is visible on chr17 (red arrow).

DNA will be subjected to solution-phase hybrid capture followed by Illumina sequencing to >100x mean coverage. Mutations and copy-number changes will be determined within the Firehose pipeline environment at the Broad Institute using a suite of existing tools, which include the published MuTect, Indelocator, GISTIC and MutSig algorithms[72,73,75-79]. To understand inter- and intratumoral heterogeneity, we will use computational tools developed by Dr. Carter to perform an integrative analysis of somatic mutations and copy-number alterations. This analysis, termed Absolute[80], estimates the cancer-cell fraction (CCF) of each mutation, allowing parsimonious resolution of the clonal architecture of bulk cancer tissue samples. Dr. Carter also developed a

computational tool called Phylogic, which automates the inference of phylogenetic trees relating all cancer subclones identified in the cancer tissue samples of each individual patient[69]. Phylogic operates by analyzing the modeled absolute copy-numbers and mutation CCF estimates provided by ABSOLUTE. **Figure 5** presents preliminary data developed by Drs. Brastianos and Carter, showing copy number alterations in the CSF of a HER2+ brain metastasis patient.

We will characterize cfDNA from CSF samples and blood at diagnosis and during treatment, and bulk DNA from primary tumors using targeted and whole-exome sequencing (WES). All samples will be subjected to targeted sequencing and selected cases of adequate quality will be subjected to WES. This will allow us to characterize somatic point mutations, insertion-deletion events, and copy-number changes.

1.3.2 MGMT Status

O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that confers resistance to TMZ. MGMT expression and activity have been determined by several methods in clinical tissues, most often by the DNA methylation status of its promoter, including methylation specific PCR (MSP), pyrosequencing and semi-quantitative MSP. Other techniques quantify total MGMT expression including IHC and QRT-PCR [81]. When tumor tissue was assessed for MGMT status using MSP, low expression of MGMT generally correlated with response or better outcome to TMZ therapy in GBM[82], TMZ combination therapy studies in GBM[83,84], and other primary brain tumors[85,86]. MGMT studies have been conducted in other cancer types when TMZ was incorporated into the therapy, including lung cancer[87], aerodigestive and colorectal cancers[88], and melanoma[89]. These studies used tumor samples from biopsy or surgery.

It is noteworthy that MGMT alterations occur in tissues other than tumor. Alterations were detected in stroma around tumor in breast cancer[90]. Similar findings have been reported for other cancers[91].The data suggest that the microenvironment may also play a role in drug sensitivity or resistance.

MGMT methylation has been measured in serum and compared to that in FFPE tumor blocks for glioma[92]. Circulating DNA was extracted from plasma using the QIAamp DNA Blood Mini kit. After bisulfite modification, an end point PCR assay, using primers specific to either methylated or unmethylated DNA, was used to determine MGMT promoter methylation status (Qiagen). The frequency of MGMT methylation was 60% in tumor and 41% in serum. Serum MGMT changed from baseline to 12 months. Both measurements predicted response to TMZ. Other studies have reported similar approaches[93-95]. CSF as a source of MGMT methylation data has only been reported in abstract form[96] and a literature search revealed no analysis of MGMT in CTCs.

In preliminary studies in the Steeg laboratory, MGMT expression was determined by IHC in FFPE blocks of matched sets of primary breast tumors and resected brain metastases (n=62)[97]. Samples were dichotomized into low expression (<5% of tumor cells+) and high expression (≥5%) categories by a pathologist. Overall concordance of primary tumor and brain metastasis MGMT expression was weak. Low MGMT expression was prevalent, in 58% of brain metastasis specimens. A strong trend was observed in brain metastases for an association of HER2 overexpression and MGMT negativity ($P = 0.089$), suggesting the eligibility of this subset for potential clinical trials.

The hypothesis to be tested in this trial is that MGMT status, either at baseline or changes over time, will reflect secondary preventive activity of TMZ. A second hypothesis posits that alterations in MGMT may be an early sign of resistance to therapy and precede progression detectable on MRI. In addition, we will determine the concordance of MGMT methylation from multiple sources (tissue, serum, CSF) and by two assays (methylation and IHC). MGMT status will be assayed from patient serum at baseline and on study, the primary FFPE tumor block as available, and CSF as available in this trial. MGMT methylation will be used for all samples. For the FFPE block, MGMT IHC will also be conducted as previously reported.

1.3.3 Neuroinflammatory response and neuronal damage metrics.

Brain metastases elicit a prominent neuro-inflammatory response including activated astrocytes, identified by GFAP positivity, and microglia[98]. Multiple preclinical studies indicate that the neuro-inflammatory milieu conducts cross-talk with tumor cells to augment metastatic colonization[99]. The hypothesis to be tested is that the extent of neuro-inflammatory response, either at baseline or through treatment, will correlate with secondary prevention efficacy of TMZ. Other goals of these studies are to determine the prevalence, magnitude and kinetics of the neuro-inflammatory response in brain metastasis progression, which has not been addressed in the clinical literature.

The neurocognitive sequelae of brain metastases derive from both the lesions and the treatments[100-103]. In preclinical studies brain metastases of breast cancer caused neuronal cell death [104], which would be a contributor to neurocognitive dysfunction. Regional or systemic tests for neuro-inflammatory responses and neurocognitive sequelae have not been meaningfully incorporated into clinical research on brain metastasis, other than mental function testing. Several leads have been developed for other brain pathologies and will be tested in this trial:

a. The traumatic brain injury (TBI) field, another disease of the brain and BBB, has pioneered blood-based assays. Blood markers of neuro-inflammation (GFAP) and neuronal injury (UCH-L1) have been measured in patients with mild-severe TBI over time[105-107]. Serum levels were measured in duplicate using an ELISA assay (Banyan Biolarmers Inc.). Levels of each protein, or a ratio of the two were reported. Normal controls or trauma patients without TBI showed low levels of each marker. Serum levels were elevated in TBI patients and correlated with several indicators of outcome, need for treatments, and disease severity. The same markers have been infrequently reported in CSF[108]. Similar results have been reported for stroke[109].

Quantification of GFAP and UCH-L1 will be performed in serum and CSF samples from all patients on the trial. Validation experiments and the serum measurements from the trial will be conducted in the Steeg laboratory. The questions to be answered include: (a) what is the variation in baseline levels of brain metastasis patients? (b) what are the changes in time with and without progression? (c) Do changes in either level, or the ratio, correlate with preventive efficacy of TMZ? (d) Do changes predict clinical progression earlier than MRI?

b. MicroRNA-34c (miR-34c) has been studied in Alzheimer's disease (AD) as a biological covariate of cognitive function[110]. miR-34c was identified as a miR enriched in the hippocampus of mice. It targets genes involved in axonogenesis, neuron projection neurogenesis, cell communication, neuronal activities, synaptic transmission, channel activity. Injection of miR-34c functionally improved learning in a mouse model of AD[110]. MiR-34c has been quantified in plasma of AD and healthy controls[111]. Of 110 AD and 123 normal control patients, RNA of sufficient integrity was extracted from 78 and 85, respectively. MiRNA extraction was completed using

plasma samples stored in RNAlater, subjected to Trizol/chloroform extraction and centrifugation. A Taqman miRNA reverse transcription kit was used to quantify miR-34c, using QPCR. A 30 ml blood sample was used, it is not known if smaller amounts can be used. Levels of miR-34c were elevated in AD patients compared to normal, or normal elderly controls, and correlated with mental function on Mini-Mental Status Examinations[111].

Preclinical work will be performed in the Steeg lab to assay validate this technique, and to determine if an amplification will permit quantification of miR-34c from smaller plasma samples. If sufficient plasma is available from patients, miR-34c levels will be determined to answer the same questions posed in (a). If sufficient miRNA is obtained, a full miRNA profile may be obtained on the samples using a miRNA array[112].

1.3.4 Exosomes

Currently there is a lack of rapid and reliable methods for cancer detection, metastatic prediction and treatment response assessment. Dr. Lyden's laboratory is developing non-invasive methods to predict patient outcome for site-specific metastasis and that can be used to monitor treatment responses. These methods are based on a simple blood test and rely on the isolation and characterization of tumor-derived exosomes from the plasma of cancer patients.

Exosomes are small membrane vesicles (30-100 nm in diameter) of endocytotic origin and secreted by most cells constitutively. These microvesicles contain cell type-specific proteins and genetic materials, including mRNAs, miRNAs and DNA, and can exert a functional influence once uptaken by recipient cells, therefore representing novel mediators of intercellular communication. Importantly, exosomes are shed from cancers and are readily isolated from the peripheral circulation. A growing body of evidence has shown that tumor-derived exosomes not only have a crucial role in regulating tumor growth and metastasis, but also provide a "treasure box" of novel non-invasive biomarkers for different types of cancers[113].

We propose to use this "liquid biopsy" in this trial to evaluate the possibility of rapidly screening for metastatic disease detection, as well as for metastasis prediction and treatment response monitoring. Analysis of exosomes in blood samples will be performed in the Lyden lab at Weill Cornell Medical College.

1.3.5 Pharmacokinetics

Both temozolomide and T-DM1 are FDA approved drugs with established information on their pharmacokinetics. Since we are using temozolomide in new dose and schedule for this study, we will sample a cohort of patients in order to determine temozolomide exposure.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Phase 1 Inclusion Criteria

2.1.1.1 Participants must have histologically confirmed HER2-positive breast cancer for which standard curative measures do not exist or are no longer effective. HER2 testing must have been performed in a laboratory accredited by the College of American Pathologists (CAP) or another accrediting entity.

2.1.1.2 Participants must have brain metastases, treated within 12 weeks of study entry with SRS,

resection or WBRT. A minimum interval of 3 weeks between completion of brain SRS and/or resection and 6 weeks for WBRT and the start of treatment in this trial will be observed to allow proper healing. The presence of concomitant extracranial metastatic disease is allowed for enrollment.

2.1.1.3 Corticosteroids will be allowed at enrollment and during the first month of treatment with T-DM1 after SRS, up to a dose of no more than 10mg of dexamethasone daily or equivalent. Participants that need to continue corticosteroids after the initial month will be allowed to continue in the protocol treatment if no further increase in dose is necessary. Participants that need increase in dose of corticosteroid after initial month will be taken off protocol treatment.

2.1.1.4 Age ≥18 years. Because breast cancer is not commonly found in pediatric population and no dosing or adverse event data are currently available on the use of temozolomide in combination with T-DM1 in participants <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

2.1.1.5 ECOG performance status ≤2 (Karnofsky ≥60%, see **Appendix A**).

2.1.1.6 Participants must have adequate organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,000/mcL
– platelets	≥100,000/mcL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	<3.0 X institutional upper limit of normal
– creatinine	up to 1.5 upper institutional limits
	OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.

2.1.1.7 Alkylating agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and for 7 months (women) or 4 months (men) after treatment completion. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.8 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Phase 1 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents.

2.1.2.2 Patients unable to speak or understand English, since they cannot complete neurocognitive evaluation.

2.1.2.3 Patients with known leptomeningeal metastatic disease.

2.1.2.4 Patients with major symptoms or impairments related to brain metastases, such as aphasia or severe confusion, will be excluded per PI discretion when those symptoms preclude proper neurocognitive evaluation during the study treatment.

2.1.2.5 Patients who have received previous treatment with T-DM1 and had systemic progression of disease while on it, are ineligible. Patients receiving treatment with T-DM1 whose only site of disease progression was brain are allowed to enroll in this trial.

2.1.2.6 Patients who have received chemotherapy in the previous 3 weeks (6 weeks for nitrosoureas or mitomycin).

2.1.2.7 HBV (HBs Ag positive) or HCV (anti-HCV positive) patients are ineligible because of potential reactivation of hepatitis virus with temozolomide use.

2.1.2.8 Grade ≥3 peripheral neuropathy according to (NCI CTCAE) version 4.0.

2.1.2.9 Cerebral Vascular Accident (CVA) or Transitory Ischemic Attack (TIA) in the year before enrollment, or presence of residual symptoms from CVA that happened more than a year before.

2.1.2.10 Pulmonary Embolism (PE) in the 3 months before enrollment. Anticoagulation is permitted.

2.1.2.11 Impaired cardiac function or clinically significant cardiac disease including the following:

- New York Heart Association grade III or IV congestive heart failure.
- Myocardial infarction within the last 12 months.
- Subjects with impaired LVEF (<50%).

2.1.2.12 Patients with inability to complete brain MRI studies with contrast.

2.1.2.13 Patients with breast tissue expanders must have those removed before enrollment.

2.1.2.14 History of allergic reactions attributed to compounds of similar chemical or biologic composition to temozolomide or T-DM1. Patients who are tolerating TDM1 successfully with premedications and slower infusion will be allowed.

2.1.2.15 Patients receiving medications that are strong CYP3A4 inhibitors or inducers are ineligible. In vitro studies indicate that DM1, the cytotoxic component of T-DM1, is metabolized mainly by CYP3A4 and to a lesser extent by CYP3A5. Concomitant use of strong CYP3A4 inhibitors with T-DM1 should be avoided due to the potential for an increase in DM1 exposure and toxicity. Consider an alternate medication with no or minimal potential to inhibit CYP3A4. Lists including medications and substances known or with the potential to interact with CYP3A4 isoenzymes are provided in **Appendix B.**

2.1.2.16 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, that would limit compliance with study requirements.

2.1.2.17 Pregnant women are excluded from this study because temozolomide is an alkylating agent with the potential for teratogenic or abortifacient effects. Because there is an

unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with temozolomide and/or T-DM1, breastfeeding should be discontinued if the mother is treated with either of those agents. These potential risks may also apply to other agents used in this study.

2.1.2.18 HIV-positive patients are excluded because these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

2.1.2.19 Patients with any other concomitant invasive malignancies are ineligible. Prior invasive cancers treated with curative intent, and with no evidence of recurrent disease, may be eligible after PI evaluation. Patients with treated limited stage basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the breast or cervix are eligible.

2.1.3 Phase 2 Inclusion Criteria

2.1.3.1 Participants must have histologically confirmed HER2-positive breast cancer for which standard curative measures do not exist or are no longer effective. HER2 testing must have been performed in a laboratory accredited by the College of American Pathologists (CAP) or another accrediting entity.

2.1.3.2 Participants must have 1-10 brain metastases, by contrast MRI, treated within 12 weeks of study entry with SRS and/or resection. A minimum interval of 3 weeks between completion of brain SRS and/or resection and the start of treatment in this trial will be observed to allow proper healing. The presence of concomitant extracranial metastatic disease is allowed for enrollment.

2.1.3.3 Corticosteroids will be allowed at enrollment and during the first month of treatment with T-DM1 after SRS, up to a dose of no more than 10mg of dexamethasone daily or equivalent. Participants that need to continue corticosteroids after the initial month will be allowed to continue in the protocol treatment if no further increase in dose is necessary. Participants that need increase in dose of corticosteroid after initial month will be taken off protocol treatment.

2.1.3.4 Age ≥18 years. Because breast cancer is not commonly found in pediatric population and no dosing or adverse event data are currently available on the use of temozolomide in combination with T-DM1 in participants <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

2.1.3.5 ECOG performance status ≤2 (Karnofsky ≥60%, see **Appendix A**).

2.1.3.6 Participants must have normal organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,000/mcL
– platelets	≥100,000/mcL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	<3.0 X institutional upper limit of normal
– creatinine	up to 1.5 upper institutional limits

OR

– creatinine clearance ≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.

2.1.3.7 Alkylating agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and for 7 months (women) or 4 months (men) after treatment completion. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.3.8 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.4 Phase 2 Exclusion Criteria

2.1.4.1 Patients who are receiving any other investigational agents.

2.1.4.2 Patients unable to speak or understand English, since they cannot complete neurocognitive evaluation.

2.1.4.3 Patients with known leptomeningeal metastatic disease.

2.1.4.4 Patients previously treated with whole brain radiation therapy (WBRT).

2.1.4.5 Patients with major symptoms or impairments related to brain metastases, such as aphasia or severe confusion, will be excluded per PI discretion when those symptoms preclude proper neurocognitive evaluation during the study treatment.

2.1.4.6 Patients who have received previous treatment with T-DM1 and had systemic progression of disease while on it, are ineligible. Patients receiving treatment with T-DM1 whose only site of disease progression was brain are allowed to enroll in this trial.

2.1.4.7 Patients who have received chemotherapy in the previous 3 weeks (6 weeks for nitrosoureas or mitomycin).

2.1.4.8 HBV (HBs Ag positive) or HCV (anti-HCV positive) patients are ineligible because of potential reactivation of hepatitis virus with temozolomide use.

2.1.4.9 Grade ≥3 peripheral neuropathy according to (NCI CTCAE) version 4.0.

2.1.4.10 Cerebral Vascular Accident (CVA) or Transitory Ischemic Attack (TIA) in the year before enrollment, or presence of residual symptoms from CVA that happened more than a year before.

2.1.4.11 Pulmonary Embolism (PE) in the 3 months before enrollment. Anticoagulation is permitted.

2.1.4.12 Impaired cardiac function or clinically significant cardiac disease including the following:

- New York Heart Association grade III or IV congestive heart failure.
- Myocardial infarction within the last 12 months.

- Subjects with impaired LVEF (<50%).

2.1.4.13 Patients with inability to complete brain MRI studies with contrast.

2.1.4.14 Patients with breast tissue expanders must have those removed before enrollment.

2.1.4.15 History of allergic reactions attributed to compounds of similar chemical or biologic composition to temozolomide or T-DM1. Patients who are tolerating TDM1 successfully with premedications and slower infusion will be allowed.

2.1.4.16 Patients receiving medications that are strong CYP3A4 inhibitors or inducers are ineligible. In vitro studies indicate that DM1, the cytotoxic component of T-DM1, is metabolized mainly by CYP3A4 and to a lesser extent by CYP3A5. Concomitant use of strong CYP3A4 inhibitors with T-DM1 should be avoided due to the potential for an increase in DM1 exposure and toxicity. Consider an alternate medication with no or minimal potential to inhibit CYP3A4. Lists including medications and substances known or with the potential to interact with CYP3A4 isoenzymes are provided in **Appendix B.**

2.1.4.17 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, that would limit compliance with study requirements.

2.1.4.18 Pregnant women are excluded from this study because temozolomide is an alkylating agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with temozolomide and/or T-DM1, breastfeeding should be discontinued if the mother is treated with either of those agents. These potential risks may also apply to other agents used in this study.

2.1.4.19 HIV-positive patients are excluded because these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

2.1.4.20 Patients with any other concomitant invasive malignancies are ineligible. Prior invasive cancers treated with curative intent, and with no evidence of recurrent disease, may be eligible after PI evaluation.

2.1.5 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. Participants will be recruited from the current patient population at NIH. The study may be listed in patient advocacy websites such as the Army of Women.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

- Minimal risk activities that may be performed before the subject has signed a consent include the following:
- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos

- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in Section **11.7**.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

Screening evaluations or tests will be done prior to enrollment within the following time frames:

Anytime:

2.2.3 Pathology confirmation of diagnosis.

Pathological confirmation of diagnosis and ER, PR and HER2 expression in the Laboratory of Pathology at NIH Clinical Center or Walter Reed National Military Medical Center at Bethesda. However, if no pathologic specimen is available, participants may enroll with a pathologist's report showing a histologic diagnosis of HER2-positive breast cancer in a College of American Pathologists (CAP) accredited laboratory and a clinical course consistent with the disease.

Within 30 days:

2.2.4 Imaging studies:

- CT of chest/abdomen /pelvis (MRI may be substituted at investigator's discretion)
- Bone scan
- Contrast enhanced brain MRI

2.2.5 Cardiology test: 2-D Echocardiogram or MUGA-scan

2.2.6 Anti-HIV, anti-HCV, Hepatitis B Surface Ag

Within 10 days:

2.2.7 History and Physical examination:

Complete medical history (including prior therapy) and physical examination (including height, weight, vital signs, ECOG performance status and complete neurological exam).

2.2.8 Laboratory Studies:

- CBC with differential,
- Biochemical profile: sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST, ALT, total bilirubin, calcium, total protein, albumin, alkaline phosphatase, magnesium.

2.2.9 Pregnancy Testing:

For female subjects of childbearing potential, urine or serum HCG will be performed. Participants who are postmenopausal (age-related amenorrhea for 12 or more consecutive months, or documented FSH > 40 mIU/mL / Estradiol < 20mIU/mL), or who had undergone hysterectomy or

bilateral oophorectomy are exempt from pregnancy testing.

For baseline evaluations, please see Section **2.5**.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

2.4 RANDOMIZATION (OR STRATIFICATION) PROCEDURES

Cohorts

Number	Name	Description
1	Phase I Cohort	Subjects on phase I portion of study
2	Phase II Cohort	Subjects on phase II portion of study

Arms

Letter	Name	Description
1	Phase I	Phase I: T-DM1 + TMZ dose escalation
2A	Phase II arm A	Phase II: T-DM1 alone
2B	Phase II arm B	Phase II: T-DM1 + TMZ

Stratifications, Randomization and Arm Assignment

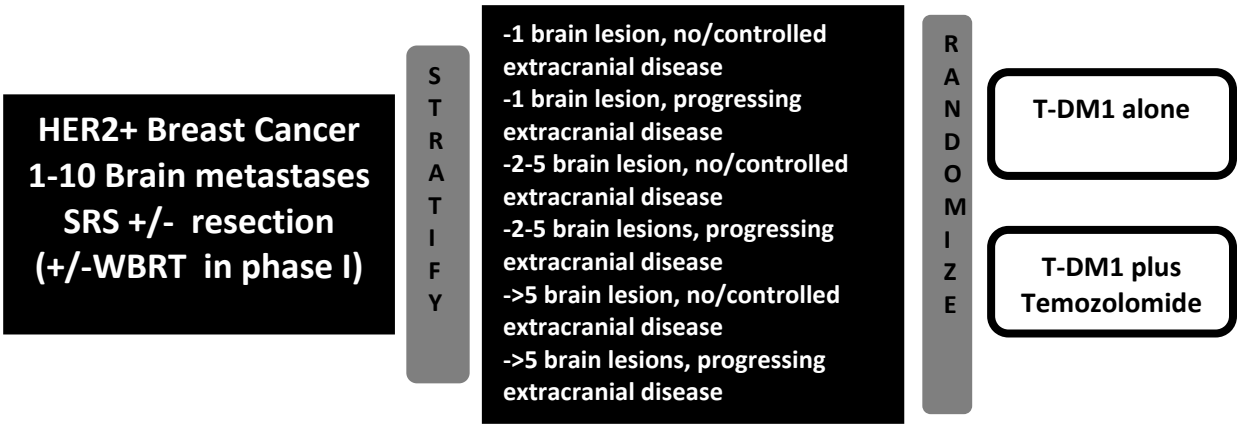
There are two stratification factors in the randomization: number of brain lesions (1 vs. 2-5 vs >5) and status of extracranial lesions (none/controlled. vs. progressing).

Name	Distinct Options
Number of CNS lesions	• 1 • 2-5 • >5
Status of extracranial lesions	• None or controlled • Progressing

This results in six stratification groups:

1. One brain lesion with no or controlled extracranial lesions
2. One brain lesion with progressing extracranial lesions
3. Two – three to five brain lesions with no or controlled extracranial lesions
4. Two – three to five brain lesions with progressing extracranial lesions
5. More than five brain lesions with no or controlled extracranial lesions
6. More than five brain lesions with progressing extracranical lesions

Within each of these six groups participants will be randomized at study entry to either arm 2A or arm 2B. Arm 2A will receive treatment with T-DM1 alone and Arm 2B will receive treatment with T-DM1 plus temozolomide. The Central Registration Office will be contacted via encrypted email at: ncicentralregistration-l@mail.nih.gov to determine the appropriate assignment for each participant. Once the NCI Central Registration Office receives subject stratification group assignment, randomization to treatment arms will be performed by the CRO.



Participants in cohort 1 will be directly assigned to arm 1.

Participants in cohort 2 will be randomized between arms 2A and 2B, stratified for number of CNS lesions and status of extracranial lesions.

2.5 BASELINE EVALUATION

The following procedures will be completed within 7 days before initiating of the treatment. Tests done at Screening do not need to be repeated on Baseline if performed in designated time frame.

2.5.1 Clinical Evaluation:

- Review of concurrent medications
- Review of baseline adverse events
- Physical exam (including complete neurological exam, vital signs, weight and ECOG performance status)

2.5.2 Laboratory Evaluation:

- CBC with differential and platelet count
- Biochemical profile: (sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST, ALT, bilirubin, calcium, total protein, albumin, alkaline phosphatase, magnesium)
- PT/INR/PTT
- TSH
- Urinalysis
- Pregnancy test for women of childbearing potential.

2.5.3 Neurocognitive and quality of life assessment:

- Baseline MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) (**Error! Reference source not found.**).
- Application of Patient-Reported Outcomes Measurement Information System (PROMIS®) cognitive questionnaire (**Error! Reference source not found.).**

2.5.4 Correlative tests evaluation:

- Blood samples for correlative studies (will be collected at Day 1 of Cycle 1).
- Lumbar puncture by Interventional Radiology for collection of CSF sample.
- Primary tumor paraffin block and /or fresh frozen tissue from previous surgery will be requested from participants at baseline to perform comparative genomic analysis as part of correlative studies planned for this clinical trial.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open label Phase I/II trial, accruing initially one cohort to determine the safety and RP2D of Temozolomide combined to T-DM1 in participants with HER2-positive breast cancer metastatic to the brain after SRS or resection of those brain metastases (Phase I); and to examine the safety and clinical activity of the Temozolomide plus T-DM1 combination versus T-DM1 alone in the same group of participants (Phase II). In the Phase II portion, participants will be randomized between the two treatment arms.

Participants will receive T-DM1 every 21-days, as per standard use recommendation. Administration of Temozolomide will be in daily dose by mouth. T-DM1 can be given up to 3 days earlier or delayed up to 14 days due to holidays, inclement weather, conflicts, or similar reasons. The timing of subsequent administrations is then adjusted to maintain a 21 day-interval.

<u>Phase I:</u>

3.1.1 Dose Limiting Toxicity

The DLT period is one cycle, 21 days. DLT is defined as any of the following:

Non-Hematologic dose-limiting toxicity:

- Grade 3 or higher adverse events (AEs), excluding:
    - Grade 3 hypertension controlled with anti-hypertensive therapy
    - Grade 3 asymptomatic electrolytes imbalance with optimal and continuing repletion that downgrades to grade 1 or better within 3 days after onset of the event.
    - Grade 3 endocrinopathy that is managed with or without hormone replacement therapy and the participant is asymptomatic.
    - Grade 3 asymptomatic increase in AST (SGOT), ALT (SGPT), that downgrades to grade 1 or better within 7 days after onset of the event.
    - Transient (lasting less than<48 hrs.) nausea/emesis/diarrhea if corrected with conservative measures within 24-48 hours.
- Persistent (>14 days) non-hematologic Grade 2 AEs despite optimal medical management and temozolomide treatment delay > 14 days.

Hematologic Toxicity:

- Grade 4 neutropenia of ≥ 7 days duration, grade 3 or 4 neutropenia of any duration with fever or documented infection (febrile neutropenia).
- Grade ≥ 3 thrombocytopenia.
- All other grade 4 hematologic toxicities, excluding:
    - Grade 4 lymphopenia, or leukopenia in the absence of grade 3 or higher neutropenia.

Note: Participants in use of corticosteroids that require increase in dose of corticosteroid after initial month of treatment in this clinical trial will be taken off protocol treatment given potential of tumor necrosis post SRS and will not be considered as DLT.

3.1.2 Dose Escalation

Dose escalation will follow the classical '3+3' trial design. If none of the first three participants in a cohort experiences a dose-limiting toxicity during one cycle of treatment, another three participants will be treated at the next higher dose level. However, if one of the first three participants experiences a dose-limiting toxicity during first cycle of treatment, three more participants will be treated at the same dose level. Dose escalation continues until the final dose level meets safety criteria or at least two participants among a cohort of three to six participants experience dose-limiting toxicities (i.e., ≥33% of participants with a dose-limiting toxicity at that dose level).

Dose Escalation Schedule		
Dose Level	T-DM1 mg/kg IV every 21 days	Temozolomide mg/m2 PO daily
Level 1	3.6	30
Level 2	3.6	40

Level 3	3.6	50

Dose escalation will follow the rules outlined in the Table below.

Number of Participants with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter up to 3 participants at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to 3 additional participants will be entered at the next lowest dose level if only 3 participants were treated previously at that dose.
1 out of 3	Enter up to 3 more participants at this dose level. • If 0 of these 3 participants experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to 3 additional participants will be entered at the next lowest dose level if only 3 participants were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended Phase II dose. At least 6 participants must be entered at the recommended Phase II dose.

During the Phase I DLT time period (first 21 days), no decrease or delay for toxicity reasons in dose of T-DM1 is allowed. If participants develop side effects considered mainly related to T-DM1 that would require dose reductions leading to discontinuation of that drug, they should be taken off protocol treatment and substituted by another participant. During the Phase II portion of this trial, dose reductions should be done as described in **3.3.1**.

Phase II:

The phase II portion of this trial will randomize participants between treatment with T-DM1 alone at standard recommended dose (3.6 mg/kg IV every 21 days) or the combination of T-DM1 at standard dose and Temozolomide. The Temozolomide recommended dose for phase II trial will be considered the maximum tolerated dose determined from the lead-in phase I trial.

3.2 DRUG ADMINISTRATION

3.2.1 T-DM1

See section **13.2.4** for preparation instructions. T-DM1 will be administered intravenously to all participants in the study. T-DM1 will be administered at standard recommended dose (3.6 mg/kg), once every 21 day cycle until disease progression, participant withdrawal or toxicities. The

infusion site will be closely monitored for possible subcutaneous infiltration during drug administration. First infusion will be administered approximately 90 minutes. Participants should be observed during the infusion and for at least 90 minutes following the initial dose for fever, chills, or other infusion related reactions. Subsequent infusions will be administered approximately 30 minutes if prior infusions were well tolerated. Participants should be observed during the infusion and for at least 30 minutes after infusion. If the infusion is interrupted, the reason for interruption will be documented in the clinical record. Administration of T-DM1 may be given 3 days earlier or delayed up to 14 days due to holidays, inclement weather, conflicts, or similar reasons. The timing of subsequent administrations is then adjusted to maintain a 21 days-interval. Participants will be monitored before and after the infusion with assessment of vital signs at the times specified in the Study Calendar (Section **3.5)**. In the event of a ≤grade 2 infusion-related reactions (IRR), the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and re-initiated at 50% of the initial rate until completion of the infusion. For subjects with a ≤grade 2 infusion related reaction, subsequent infusions may be administered at 50% of the rate at which the IRR occurred. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications may be administered at the discretion of the investigator. T-DM1 will be given until disease progression, participant withdrawal or toxicities. The start of the first cycle will be scheduled within 7 days of registration. In the event of G3 or G4 IRR, treatment should be stopped and not repeated in the future.

3.2.2 Temozolomide

During Phase I all participants will receive temozolomide and T-DM1. During Phase II only participants randomized to the combination treatment arm, will receive temozolomide and T-DM1. Temozolomide at the appropriate dose level will be given orally continuously once a day, every day during the 21 cycle. Temozolomide will be given in combination with T-DM1 until disease progression, participant withdrawal or toxicities. Temozolomide will be dispensed at the start of each cycle. Participants will be provided with a pill diary (**Appendix C**), instructed in its use, and asked to bring it with them to each appointment. The dose will be determined using the body surface area (BSA) calculated at the beginning of each cycle unless significant (> 3 kg) weight loss or gain is observed. The BSA will be calculated from the height obtained at the pretreatment visit and from the weight obtained at the visit before each odd cycle. Capsules of temozolomide will be available in 5, 20, and 100 mg for this study. The daily dose will be rounded to the nearest 5 mg. The exact dose administered should be recorded in the CRF. Each daily dose should be given with the least number of capsules. Prior to each treatment cycle with temozolomide a complete blood count (CBC) will be obtained (within 72 hours prior to dosing).

3.2.3 Self-Administered Study Drugs

The Temozolomide used in this study is a self-administered investigational agent. Such agents are dispensed from the pharmacy to a participant or to a Patient Care Unit for self-medication and a record of the dispensed investigational agent is generated and kept by the dispensing pharmacy.

Participants will be instructed to fast at least 2 hours before and 1 hour after temozolomide administration. Water is allowed during the fast period. Participants will be instructed to swallow the capsules whole, in rapid succession, without chewing them. If vomiting occurs during the course of treatment, no re-dosing of the participant is allowed before the next scheduled dose. Antiemetic prophylaxis with a 5-HT3 antagonist is strongly recommended and should administered 30 to 60 minutes before temozolomide administration.

As indicated above, participants will be asked to keep a medication diary and bring it with them on each study visit. Participants will also bring any remaining pills to each study visit. The Research Nurse reviews and validates the completeness and accuracy of the participant's diary with the participant.

If a participant goes off study while at home, the Research Nurse will ensure and document the return of the unused oral investigational agents from the participant. Unused investigational agent will be destroyed per dispensing pharmacy procedure.

3.3 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITIES:

- In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).
- Once a participant has a dose reduction for toxicity, the dose will not be increased.
- Participants continuing to experience toxicity at the off-study visit will be contacted for additional assessments until the toxicity has resolved or is deemed irreversible. Participants must remain on the study to have additional assessment.
- For AEs that are unrelated to the study drugs, study drug may be held for up to 14 days at the discretion of the PI.

3.3.1 T-DM1 Dose Modifications:

If a planned dose is delayed or missed, administer as soon as possible; do not wait until the next planned cycle. Adjust the schedule of administration to maintain a 3-week interval between doses. Administer the infusion at the dose and rate the participant tolerated in the most recent infusion.

Management of increased serum transaminases, hyperbilirubinemia, left ventricular dysfunction, thrombocytopenia, pulmonary toxicity or peripheral neuropathy may require temporary interruption, dose reduction or treatment discontinuation of T-DM1 as per guidelines provided in the tables below.

Recommended dose reductions for AEs during Phase II part of the trial.

Dose level	Dose
Starting dose	3.6 mg/kg
Dose reduction -1	3.0 mg/kg
Dose reduction -2	2.4 mg/kg
Stop treatment	No treatment

Dose Modification Guidelines for Infusion Related Reactions

Grade ≤ 2	Grade ≥3
Reduce rate of infusion to 50% of the rate at which the toxicity occurred	Permanently discontinue T-DM1

Dose Modification Guidelines for Increased Serum Transaminases (AST/ALT)

Grade 2 (< 3 to ≤ 5 X ULN)	Grade 3 (< 5 to ≤ 20 X ULN)	Grade 4 (>20 X ULN)
Treat at same dose level	Do not administer T-DM1 until AST/ALT recovers to Grade ≤ 2, and then reduce one dose level.	Permanently discontinue T-DM1

ALT = alanine transaminase; AST = aspartate transaminase; ULN = upper limit of normal.

Dose Modification Guidelines for Hyperbilirubinemia

Grade 2 (>1.5 to ≤ 3 X ULN)	Grade 3 (>3 to ≤ 10 X ULN)	Grade 4 (>10 X ULN)
Do not administer T-DM1 until total bilirubin recovers to Grade ≤ 1, and then treat at same dose level.	Do not administer T-DM1 until total bilirubin recovers to Grade ≤ 1, and then reduce one dose level.	Permanently discontinue T-DM1.

Permanently discontinue T-DM1 treatment in participants with serum transaminases > 3 x ULN and concomitant total bilirubin > 2 X ULN.
Permanently discontinue T-DM1 in participants diagnosed with nodular regenerative hyperplasia (NRH).

Dose Modifications for Left Ventricular Dysfunction

Symptomatic CHF	**LVEF <40%**	**LVEF 40% to ≤ 45% and decrease is ≥ 10% points from baseline**	**LVEF 40% to ≤ 45% and decrease is < 10% points from baseline**	**LVEF >45%**
Discontinue T-DM1	Do not administer T-DM1. Repeat LVEF assessment within 3 weeks. If LVEF < 40% is confirmed, discontinue T-DM1.	Do not administer T-DM1. Repeat LVEF assessment within 3 weeks. If the LVEF has not recovered to within 10% points from baseline, discontinue T-DM1.	Continue treatment with T-DM1. Repeat LVEF assessment within 3 weeks.	Continue treatment with T-DM1

CHF = Congestive Heart Failure; LVEF = Left Ventricular Ejection Fraction

Dose Modification Guidelines for Thrombocytopenia

Grade 3	**Grade 4**
PLT 25.000/L to <50.000/L	PLT <25.000/L
Do not administer T-DM1 (hold up to 4 weeks) until platelet count recovers to ≤ Grade 2 (50,000/L to < 75,000/L) , and then treat at same dose level, or reduce one dose level at the discretion of the investigator. If not resolved after 4 weeks, then stop.	Do not administer T-DM1 (hold up to 4 weeks) until platelet count recovers to ≤ Grade 2 (50,000/L to < 75,000/L), and then reduce one dose level. If not resolved after 4 weeks, then stop.

PLT = platelets

Dose modification for Interstitial Lung Disease:

Monitor participants for signs and symptoms of pneumonitis or ILD (new onset or worsening

shortness of breath or cough). Participants should be evaluated with imaging and/or pulmonary function tests including other diagnostic procedures as described below. Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up and high-resolution CT scan. Permanently discontinue T-DM1 in participants diagnosed with interstitial lung disease (ILD) or pneumonitis.

Dose modification for Peripheral Neuropathy:

Temporarily discontinue T-DM1 in participants experiencing Grade 3 or 4 peripheral neuropathy until resolution to ≤ Grade 2.

3.3.2 Temozolomide Dose Modifications

Dosing is based on adverse events (AEs) during the prior treatment cycle. If multiple AEs are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single AE.

Dose Level	Dose mg/m2 daily	Comments
0	RP2D	Starting dose for cycle 1 of phase II
-1	RP2D -10	Reduction if prior AE
-2	RP2D -20	Reduction if prior AE

Temozolomide will be started at a dose recommended for phase 2 after completion of phase 1 portion of this study.

Planned delay:

On day 1 of each cycle (within the prior 72 hours), if ANC ≤ 1.0 x 10^9 /L, platelet count ≤ 75 x 10^9 /L and until all grade 2, 3 or 4 non-hematologic AEs (except for alopecia, nausea, and vomiting) have resolved to grade ≤ 1.

If AEs persists, treatment should be delayed by 1 week for up to 4 consecutive weeks. If, after 4 weeks of delay, all treatment related AEs have still not resolved (to grade ≤ 1): then any further treatment with temozolomide should be stopped.

Dose reductions: If any treatment related non-hematologic AE observed was grade > 2 (except alopecia, lymphopenia, nausea and vomiting) and/or if platelets < 50 x 10^9 /L and/or ANC < 1 x 10^9 /L, then the dose should be reduced by one dose level. Participants who require more than two dose reductions will have treatment stopped. If any treatment-related non-hematologic AE observed was grade 4 (except alopecia, lymphopenia, nausea and vomiting) then temozolomide treatment should be stopped.

Any dose reductions of temozolomide will be determined according to: (1) non-hematologic AE during the preceding treatment cycle, as well as (2) the lowest ANC and platelets observed. No dose escalation should be attempted.

Important: If the dose was reduced or delayed for AEs, there will be no dose re-escalation in subsequent treatment cycles.

Summary of Dose Modifications or Discontinuation for Temozolomide-Related Adverse Events

Worst Treatment-Related Non-Hematologic AE (except for alopecia, nausea, and vomiting) During the Previous Cycles	
Grade	Dose modification
0-2	No dose modifications for non-hematologic AEs. Dose reductions based on ANC and platelet counts are applicable.
3	Reduce by one dose level (except alopecia, nausea, and vomiting).
4	Stop (except alopecia, nausea, and vomiting). Dose modifications based on ANC and platelet counts are not applicable.

Worst Treatment-Related Hematologic AE During the Previous Cycle

Worst AE		Platelets		
		≥75.000 /L	50 – 75.000 /L	< 50.000 /L
ANC	≥ 1.5 x 10^9 /L	Dose unchanged	Dose unchanged	Reduce 1 dose level
	≥1 & ≥ 1.5 x 10^9 /L	Dose unchanged	Dose unchanged	Reduce 1 dose level
	< 1 x 10^9 /L	Reduce 1 dose level	Reduce 1 dose level	Reduce 1 dose level

Note: A complete blood count must be performed on days 14 and 28 (± 72 hours) after the first daily dose of each treatment cycle.

Hematologic AE on Day 1 of Each Cycle (within the prior 72 hours before Day 1)	
AE	Delay
ANC< 1.000/L	Delay up to 4 weeks until resolved. If not resolved after 4 weeks, then stop. If resolved, dose delay/reductions based on non-hematologic AEs are applicable.
Platelet count < 50,000 /L	Delay up to 4 weeks until resolved to grade 2 or better (≥ 50,000 /L), then continue with same dose, or ability to dose reduce per investigator discretion. If resolved, dose delay/reductions based on non-hematologic AEs are applicable. If not resolved after 4 weeks, then stop.

Non-Hematologic AE (except for alopecia, nausea, and vomiting) On Day 1 of Each Cycle (within the prior 72 hours)	
Grade	Delay
2-4	Delay up to 4 weeks until all resolved (to grade ≤ 1). If not resolved after 4 weeks, then stop. If resolved, dose delay/reductions based on ANC and platelets are applicable.

Instructions on Missed Doses:
Participants should be instructed to take the medication as soon as it is remembered.

The next due dose should be taken no sooner than 12 hours after the previous dose, and the time of administration of the subsequent doses should be adjusted until it is back on its regular “am or pm” schedule.

3.4 QUESTIONNAIRES

The MDASI-BT (**Error! Reference source not found.**) and the PROMIS® (**Error! Reference source not found.**) will be utilized for this portion of the study. Full instruments are provided in the appendix. Both questionnaires will be filled by the participants at the same timepoint where they have scheduled brain MRIs scheduled, before the appointment with the physician. In addition, information regarding demographics and treatment history will be collected as part of the larger study and used in this analysis.

The MDASI-BT consists of 23 symptoms rated on an 11-point scale (0 to 10) to indicate the presence and severity of the symptom, with 0 being "not present" and 10 being "as bad as you can imagine." Each symptom is rated at its worst in the last 24 hours. Symptoms included on the instrument include those commonly associated with cancer therapies, those associated with increased intracranial pressure, and those related to focal deficits. The questionnaire also includes ratings of how much symptoms interfered with different aspects of a participant's life in the last 24 hours. These interference items include: general activity, mood, work (includes both work outside the home and housework), relations with other people, walking, and enjoyment of life. The interference items are also measured on 0 - 10 scales. The average time to complete these instruments is 5 minutes. The MDASI-BT or SP has been translated into 18 languages [114].

The PROMIS® assess patient-perceived functional abilities with regard to cognitive tasks including the perception that one's cognitive ability with regard to the domain of inquiry (e.g. concentration, memory) has not changed. (http://www.healthmeasures.net/explore-measurement-systems/promis) The applied cognition abilities is universal rather than disease specific. Each question has five response options ranging in value from one to five. To find the total raw score for a short form with all questions answered, sum the values of the response to each question. Using rigorous methods, PROMIS® has developed item banks (a collection of items that assess a specific trait, e.g., pain). These instruments are available to researchers free of cost. PROMIS® can be applied as computerized tests or on paper and have been translated into languages other than English [115].

3.5 STUDY CALENDAR

Procedure	Screening [15]	Baseline	Day 1 of each Cycle[13, 15]	End of treatment visit[14]	Long Term Follow-up
T-DM1[1]			X		
Temozolomide[2]			X		
History	X				
Pathology confirmation	X				
Height	X				
PE[3]	X	X	X		
NIH Advanced Directives Form[4]		X			
HIV, HCV, HepB	X				
Cardiology Assessments at screening and every 3 months (ECHO)[5]	X		X		
CT of chest/abdomen /pelvis and Brain MRI[6]	X		X		
Bone scan	X				
CBC with differential	X	X	X		
Biochemical profile [7]	X	X	X		
CD4 T cell count			X		
PT/INR/PTT [17]		X	X		
TSH		X	X		
Urinalysis		X	X		
Pregnancy testing[8]	X	X	X	X	
Concomitant Medications		X	X		
Adverse events		X	X	X	
MDASI-BT assessment[9]		X	X		
PROMIS[9]		X	X		
Blood samples for Correlative Research Studies			X[16]		
Tumor block[10]		X			
Fresh frozen tissue[11]		X	X		
CSF sample / lumbar puncture by IR[12]		X	X		
Phone call or e-mail for survival/new cancer treatments every 3 months once off treatment					X

[1] Participants will receive 3.6 mg/kg T-DM1 IV on Day 1 of each cycle. One cycle is 21 days. Allowance for scheduling changes: Brief interruption and delay in the 21-day cycle (- 3/ +14 days) may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts and government holidays, etc. This can also extend to complications of disease not attributable to disease progression or protocol therapy. These delays will not be considered protocol deviation.

[2] During Phase I all patents will receive Temozolomide in a dose according to escalation schedule from 30 to 50 mg/m2 PO daily. During Phase II patents, randomized to Arm B will receive Temozolomide according to dose estimated during Phase I

[3]Weight, vital signs, ECOG performance status and complete neurological exam

[4] As indicated in section **11.3**, all subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required

[5] Cardiology assessments: 2-D Echocardiogram or MUGA-scan; to be repeated every 3 months (+/- 2 weeks to adjust to participant visits)

[6] During Year 1, CT of chest/abdomen/pelvis and contrast brain MRI will be repeated every 6 weeks (- 3/ +14 days). Beginning with Year 2, imaging may be completed every 12 weeks (- 3/ +14 days) at the discretion of the PI.

[7] Sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST, ALT, bilirubin total and direct, calcium, total protein, albumin, alkaline phosphatase, magnesium

[8] Urine or serum HCG

[9] On baseline and every Odd Numbered Cycle, to be completed within +/- 1week of the MRI assessment date. Optional for people who do not speak English.

[10] Primary tumor paraffin block or unstained slides (minimum of 20 slides) will be requested at enrollment to perform comparative genomic analysis as part of correlative studies planned for this clinical trial.

[11] Only applies to participants that have undergone tumor resection at recurrence or prior to study enrollment.

[12] LP guided by fluoroscopy at Baseline and C3D1 are mandatory, three LP guided by fluoroscopy after C3 are optional.

[13] Tests done at Baseline do not need to be repeated on Day 1 of Cycle 1 if performed during 72 hours before C1D1. Tests can be done 72 hours before Day 1 of every cycle.

[14] End of treatment visit will occur approximately 30 days after the last dose of study drug. If the participant cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs from a local physician or laboratory. If this is not possible, participants may be assessed by telephone for symptoms.

[15] Screening and Day 1 cycle visits for Cycle 3 and beyond may be completed by remote visit with a member of the study team (e.g., if the participant is not able to return to the NIH CC). Remote visits will be conducted in compliance with NIH guidelines and FDA regulations. A participant may be referred to their local provider for pre-treatment assessment, T-DM1 IV treatment, and lab tests, or asked to come to the NIH CC for an in-person assessment, if clinically indicated, and at the discretion of the PI. In the case of any visits with participants' local providers, records will be obtained. There is no variation among labs for tests being performed; they are all standardized tests. In the event that the participant is unable to return to the NIH Clinical Center during a follow up visit, imaging may be delayed, but will be completed

as soon as feasible. Collection of research blood, CSF or urine will be omitted or collected in a delayed fashion.

[16] Pharmacokinetics samples will be collected one time only, Phase I only. See Section **5.1**.

[17] Every effort will be made to collect PT/ INR and aPTT baseline and as clinically indicated. Routine testing may resume when tubes become available.

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

Participants will not be compensated on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

- Progressive disease (distant brain metastases or systemic disease progression)
- Necessity to increase dose of corticosteroid
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in sections **3.1** and **3.3**
- Investigator discretion
- Positive pregnancy test

3.7.2 Off-Study Criteria

- Participant requests to be withdrawn from study
- Death
- Investigator discretion
- PI decision to end the study
- Screen failure
- Lost to follow-up

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 4 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 14 days, counsel the participant on the importance of maintaining the assigned visit schedule. and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 T-DM1

4.1.1 Drug Interactions

No formal drug-drug interaction studies with T-DM1 have been conducted. *In vitro* studies indicate that DM1, the cytotoxic component of T-DM1, is metabolized mainly by CYP3A4 and to a lesser extent by CYP3A5. Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) with T_DM1 should be avoided due to the potential for an increase in DM1 exposure and toxicity (**Appendix B**). Consider an alternate medication with no or minimal potential to inhibit CYP3A4. If concomitant use of strong CYP3A4 inhibitors is unavoidable, consider delaying T-DM1 treatment until the strong CYP3A4 inhibitors have cleared from the circulation (approximately 3 elimination half-lives of the inhibitors) when possible. If a strong CYP3A4 inhibitor is coadministered and T-DM1 treatment cannot be delayed, participants should be closely monitored for adverse reactions.

4.2 TEMOZOLOMIDE

4.2.1 Drug Interactions

Administration of valproic acid decreases oral clearance of temozolomide by about 5% and should be avoided in this study participants.

4.2.2 General

All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol and documented on each site's source documents as concomitant medication.

Platelet support: Prophylactic use of platelet is only used when Platelet count < 20 x 10^9 /L. Platelet transfusion is provided for active bleeding participants with thrombocytopenia to keep platelet count above 50 x 10^9 /L.

Corticosteroids: Corticosteroids will be allowed at enrollment and during the first month of treatment with T-DM1 after SRS, up to a dose of no more than 10 mg of dexamethasone daily or equivalent. Participants that need to continue corticosteroids after the initial month will not be allowed to increase the dose after that period. Participants requiring dose increase will be off protocol.

G-CSF administration: Routine prophylactic use is not permitted. However, therapeutic use in participants with complications (severe neutropenia with fever), may be considered at the investigator's discretion.

Febrile Neutropenia: It may be managed according to the local institution's Infectious Disease guidelines. Measures may include appropriate laboratory testing, including blood and urine cultures and the institution of broad-spectrum antibiotics. If a source for the fever is not identified or the fever resolves when the neutrophil count recovers, antibiotics may be discontinued and the participant observed.

Pneumocystis Pneumonia Prophylaxis: recommended to participants in use of temozolomide and CD4 counts <200/mm^3.

Antiemetics: Prophylactic and therapeutic use is allowed. Other concomitant medications and the therapies considered necessary for the well-being of the participant may be given at the discretion of the treating physician. All concomitant medications must be recorded.

Bisphosphonates, regardless of indication, provided subjects have been on stable doses for at least 2 weeks prior to randomization. Stable dose should be maintained during the investigational product treatment period. Subjects requiring initiation of bisphosphonate treatment, during the course of this study, should be discontinued due to progressive disease unless disease progression can be completely ruled out and clearly documented in the subject's source documentation.

Other anticancer experimental therapies: No other anticancer therapy (including chemotherapy, radiation, hormonal treatment or immunotherapy) of any kind is permitted during the study period. No other antitumor drugs under investigation may be used concomitantly with the study drug.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

Correlative studies rationale is discussed in detail in section **1.3** of this protocol. Studies will address aspects of TMZ resistance, molecular alterations in brain metastatic tumor cells, the neuro-inflammatory response, and markers of cognition. Biospecimen collection and storage protocols are summarized below.

Test/assay	Volume (approx.)	Type of tube***	Collection point	Location of specimen analysis
Whole genome sequence	Tumor sample		if participant undergoes surgery immediately before enrollment or at the time of disease progression. Primary tumor paraffin block or unstained slides	Brastianos Lab at MGH or Carter Lab at Harvard
MGMT Analysis	Tumor sample		if participant undergoes surgery immediately before enrollment or at the time of disease progression. Primary tumor paraffin block or unstained slides	Steeg Lab at NCI
MGMT Analysis	CSF, 2 ml	2 ml in plypropylene tubes	C1D1, C3D1, every Odd Numbered Cycle after C3*	Steeg Lab at NCI
Cell free DNA for sequencing	CSF, 10 ml	Streck Cell-Free DNA Blood Collection Tube (BCT	C1D1, C3D1, every Odd Numbered Cycle after C3*	Brastianos Lab at MGH or Carter Lab at Harvard
Neuroinflammatory marker analysis	CSF, 2 ml	2 ml in polypropylene tubes	C1D1, C3D1, every Odd Numbered Cycle after C3*	Steeg Lab at NCI
Cell free DNA for sequencing	Plasma, 10 ml	Two Streck Cell-Free DNA BCTs or BD Vacutainer EDTA tubes	D1 of each cycle	Brastianos Lab at MGH or Carter Lab at Harvard
MGMT analysis	Plasma, 10 ml	Two Streck Cell-Free DNA BCTs or BD Vacutainer EDTA tubes	D1 of each cycle	Steeg Lab at NCI

Test/assay	Volume (approx.)	Type of tube***	Collection point	Location of specimen analysis
Exosome analysis	Plasma, 5-10 ml	EDTA (purple top) tubes	D1 of each cycle	Lyden Lab at Weill Medical College
Neuroinflammatory marker analysis	Blood, 10 ml	Serum separation tube without separator gel	D1 of each cycle	Steeg Lab at NCI
MicroRNA-34c (miR-34c)	Blood, 5 ml	Serum separation tube without separator gel	D1 of each cycle	Steeg Lab at NCI
PK samples	Blood, 3 ml	Sodium heparin collection tubes (green top)	Pre dose,1, 2, 3, 4 and 8 hours post Temozolomide **	Figg Lab at NCI

*CSF at Baseline and C3D1 are mandatory, every Odd Numbered Cycle after C3 is optional
**PK blood will be collected in a sample of the phase I study subjects in order to determine exposure to temozolomide, in one day, at schedule determined in the table. In this instance, PK data will be collected in the C1 PK CRF regardless of the time point of collection.
*** Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.2 SAMPLE COLLECTION

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material

type, as well as box and freezer location. Participant demographics associated with the clinical center participant number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.1 Samples will be coded, linked in the BPC and sent for final analysis to:

Brastianos Lab
Priscilla K. Brastianos, MD
Central Nervous System Metastasis Program
Division of Neuro-Oncology, Massachusetts General Hospital
55 Fruit Street, Yawkey 9E
Boston, MA 02115
Phone: 617-643-1938
Fax: 617-643-2591
Email: pbrastianos@mgh.harvard.edu

Lyden Lab
David C. Lyden, MD, PhD
Stavros S. Niarchos Professor
Department of Pediatrics, and Cell and Developmental Biology
Weill Medical College of Cornell University
413 E. 69th Street, Box 284
New York, NY 10021
Phone: 646-962-6238
Fax: 646-962-0574
e-mail: dcl2001@med.cornell.edu

Steeg Lab
1. Patricia S. Steeg, Ph.D.
    a. Email: steegp@mail.nih.gov
    b. Phone: 301-402-2732
2. Brunilde Gril, Ph.D.
    a. Email: grilbrun@mail.nih.gov
    b. Phone: 301-451-6445

Building 37, Room 1126
NIH, Bethesda, MD 20892

5.2.2 Tumor, Blood Samples and CSF samples:

Fresh frozen brain tumor tissue will be requested from participants undergoing surgery immediately before enrollment or at the time of disease progression.

The slides and tissue blocks are stored indefinitely. All specimens are catalogued and retrieved utilizing the laboratory information systems. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the participant was enrolled.

Blood samples will be collected at Day 1 of each cycle.

CSF, guided by fluoroscopy done at Baseline and C3D1 are mandatory, every Odd Numbered Cycle after C3 is optional.

Effective with Amendment (version date 07/12/2021): PK blood samples will be collected per designated time points (pre dose, 1, 2, 3, 4, and 8 hours post Temozolomide) in one day only, in a sample of the participants remaining in the Phase I portion of the study.

The collection will follow as: collect 3mL of blood into 4ml (green top) sodium heparin collection tubes. Mix by gently inverting 6 times. Place the tubes immediately on wet ice-water bath after collection. Centrifuge samples within 1 hour after collection at 1300 x g for 10 minutes at 4°C. Place the centrifuged tubes on wet ice. Separate the plasma from the blood into a labeled polpropylene freezer vial. The samples should be processed to plasma within 30 minutes from centrifugation and the pH adjusted to <4 with the use of 8.5% phosphoric acid (15µL of 8.5% phosphoric acid per 0.5mL of plasma). Plasma should then be stored frozen at -70°C until subsequent batch analysis. The collection and analysis will be perfomed by the Figg lab at NCI.

5.2.2.1 MGMT methylation in tumor and blood samples.

MGMT methylation will be tested at protein level by IHC and at mRNA level by methylation-dependent PCR in the Steeg laboratory. For DNA methylation analysis, a procedure previously published will be utilized [92]. For IHC, the previously published procedure will be utilized [57].

5.2.2.2 Neuroinflammatory response and neuronal damage metrics in tumor and blood samples.

Analysis of GFAP, UCH-L1 and MicroRNA-34c (miR-34c) levels in serum and CSF will be performed by the Steeg Lab.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.3.1 Participant sample protections

Each participant sample set will be coded with a unique participant identifier. No participant specific information is encoded in this identifier. The protocol scientific investigators handling samples will be blinded as to as to the participant identification, participant data and outcome.

The amount of blood that may be drawn from adult participants (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

5.3.2 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20° or -80° C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research

purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if so requested). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the participant withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Description of the scope of genetic/genomic analysis

- Tumor whole-exome sequencing will be performed in collaboration with the Brastianos and Carter' laboratories at Harvard.
- Tumor cfDNA and primary tumor DNA sequencing in collaboration with Brastianos and Carter Laboratories
- We plan to characterize cfDNA from CSF samples and blood at enrollment and during treatment. All samples will be subjected to targeted sequencing and selected cases of adequate quality will be subjected to WES. This will allow us to characterize somatic point mutations, insertion-deletion events, and copy-number changes.
- Exosomes analysis in collaboration with Dr. Lyden's laboratory at Weill Cornell Medical College.

5.4.2 Privacy and Confidentiality of medical information/biological specimens

Fresh tumor and blood samples will be stored in a minus 80-degree freezer. The samples of each participant will be barcoded. At no time will participant's names be used on the blood and tissue samples. Sometimes, because a group collaboration or journal policy requires it, a subject's genetic data may be deposited in a database such as dbGaP. Although there is controlled access to such a

database, such a submission carries theoretical risks of revealing the identity of the subject. This is discussed in the consent.

5.4.3 A Certificate of Confidentiality will be obtained for the study as described in section **11.5.12**.

5.4.4 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory.

5.4.5 Genetic counseling

If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling with the NCI Genetics Branch to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study treatment, Study Day 1, through 30 days after the subject received the last administration of the study agent.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Source Documents

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information, patients' diaries or evaluation checklists, pharmacy dispensing and other records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, X-rays. The investigator will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s), providing direct access to source documents.

6.1.2 Case Report Forms

Data may be entered from the source documents directly into eCRFs for each participant enrolled in this study. The principal investigator or research nurse will review the eCRFs for completeness and accuracy. Independent audits may also be conducted by NCI personnel to ensure completeness and accuracy of data in study database.

6.1.3 Data Quality Assurance

The research team will monitor each participant's dataset throughout the study. Source document review will be made against entries on the eCRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and regulations. In addition, after the research team (data managers) completes the CRFs a research nurse or physician at the NCI will review and verify the data.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Coded, linked or identified data with approved outside collaborators under appropriate agreements.

Data will be shared through*:*

- An NIH-funded or approved public repository: clinicaltrials.gov.
- BTRIS (automatic for activities in the Clinical Center)

- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

Data will be shared:

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the purposes of this study, participants should be re-evaluated for response every 6 weeks. In addition to baseline images, confirmatory images should also be obtained 3 weeks following initial documentation of objective response.

6.3.1 RANO-BM Criteria for Evaluation of Brain Lesions

The primary endpoint of this study, however, is 12-month freedom from distant brain metastases after initial SRS or surgery. A combination of the neurological examination and MRI brain scan will be used to define progression. Due to improvements in neuroimaging and the fact that tumor growth in certain regions of the CNS is without immediate neurologic signs and symptoms, greater reliance is placed on neuroimaging to define progression. Brain MRI assessment for CNS disease progression will be done every 6 weeks. Time to distant brain CNS failure will be scored from the date of randomization to the date of diagnosis of a new focus of contrast enhancement outside the prior SRS region. Any lesions that cannot be undoubtedly classified as new or increasing tumor will have to be confirmed to be present on two consecutive MRI scans and growing, following recommendations of The Response Assessment in Neuro-Oncology Brain Metastases (RANO-BM) [116]. (**Appendix D)**

Participants with questionable progression or recurrence of tumor in areas of brain previously treated by SRS, that cannot be differentiated from necrosis post SRS without biopsy, will be continued in protocol treatment and follow-up, as long as no local treatment is recommended by their primary physicians (oncology or radiation therapy). In the instance wherein a lesion is found retrospectively and pre-dated study therapy, that lesion or lesions may receive local treatment if deemed in the best interest of the partcipant. Participants indicated to receive WBRT or new SRS because of local recurrence in brain will be off protocol and censored for analysis. Participants with systemic progression with no CNS new lesions will also be off protocol and censored for analysis. Participants with new brain tumor lesions away from previously treated (by SRS or surgery) are the ones considered progression of disease, independently of the systemic status of disease and/or concomitant recurrence in brain areas previously treated by SRS/surgery. They will be off protocol for progression with distant brain metastases and will be accounted for in the statistical analysis.

6.3.1.1 Definitions of Response

Measurable Disease:

Bi-dimensionally measurable lesions with clearly defined margins by MRI scan.

Objective Status, To Be Recorded at Each Evaluation:

If there are too many measurable lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study. The remaining lesions will be considered evaluable for the purpose of objective status determination. Unless progression is observed, objective status can only be determined when ALL measurable and evaluable sites and lesions are assessed.

Response Criteria

Stable/No Progression: No evidence of new measurable lesions on MR imaging. The designation of Stable/No Progression requires a minimum of 12 weeks duration. All measurable and evaluable sites must be assessed using the same techniques as baseline.

Partial response: ≥ 50% reduction in the sum of products of all measurable lesions over baseline sum observed using the same techniques as baseline. The patient must be on a stable or decreased dose of corticosteroids to be evaluable for response.

Complete response: Complete resolution of all lesions. The patient cannot be on any corticosteroids with the exception of adrenal replacement doses.

Progression: 25% increase in the sum of products of all measurable lesions over smallest sum observed (over baseline if no decrease) using the same techniques as baseline, OR clear worsening of any evaluable disease, OR appearance of any new lesion/site, OR failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer).

Unknown: Progression has not been documented and one or more measurable or evaluable sites have not been assessed.

6.3.2 RECIST for Systemic Evaluation

Systemic response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

If participant develop additional systemic lesions without CNS progression and require new therapy, they will be taken off the trial. This group will be followed separately to determine their RFS. Additional participant will be accrued to meet the 49 goal with one year of follow up.

6.3.2.1 Definitions

Evaluable for toxicity: All participant will be evaluable for toxicity from the time of their first treatment with Temozolomide and/or T-DM1.

Evaluable for objective response: Only those participant who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participant will have their response classified according to the definitions stated below. (Note: Participant who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Participant who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle

of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be

recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be

guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.2.4 Response Criteria

6.3.2.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.2.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.2.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participant with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Participant with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participant with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

6.3.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.4 Relapse -Free Survival

RFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.3.5 Validation of Disease Assessment (Safety Mechanisms)

The following practices will ensure accuracy in disease assessment:

Dr. Ritu Shah, Neuroradiolgist, or her designee will be provided with a list of patients scheduled for imaging by a member of the WMB team approximately 4-5 days in advance of clinic except in cases where urgent imaging is warranted. In the case of urgent scans, a member of the WMB team will make every effort to contact Dr. Shah with as much notice as the clinical situation allows prior to and/or after the urgent imaging as been performed.

The electronic medical record (EMR) protocol order set for Brain MRIs has been modified to include the following language provided by the Neuroradiology Leadership, "Comment on any new lesions relative to baseline. Any enhancing lesion 5 x 5mm or greater described for the FIRST time is to be called as a NEW lesion, even if it can be seen in retrospect on older studies. For comparison, use Baseline scan of _____." Should additional clarity or requests be made by Neuroradiology regarding order language, we will continue to amend the orders in a timely manner. Every effort will be made that each brain MRI will be initially read by neuroradiologist on call the day scans are performed or on the day patient(s) present to clinic; this review is to rule out any major finding requiring immediate attention. To review specific imaging findings related to the study, Dr. Shah will meet with Dr Zimmer to review the images within 7 days, optimally within 24-48 hours. Dr. Zimmer will document the patient respective findings in the EMR then route for Dr. Shah's co-signature. In the absence of Dr. Shah, she may delegate her responsibilities to a Neuroradiology colleague.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to the Clinical Director/designee at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet or have a teleconference every other week when participants are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Data Safety Monitoring Board (DSMB)

This protocol requires monitoring by the NCI CCR Data Safety Monitoring Board (DSMB) as described in Section **9.4.6**. Interim outcome results will not be revealed to the investigators of the trial; results will be presented to the investigators prior to final accrual to the trial only if the DSMB recommends early termination of the trial.

7.4.3 Corrective Action Plan

The study has implemented a corrective action plan to assure timely and accurate disease assessment. Please reference Section **6.3.5** Validation of Disease Assessment (Safety Mechanisms).

Additionally, the study will undergo oversight by an Independent Monitoring Committee (**7.4.4**).

7.4.4 Independent Monitoring Committee (IMC)

Additional oversight for this study will be provided by an Independent Monitoring Committee consisting of the Principal Investigator and two qualified investigators who are independent of the study team. The committee meetings will be conducted annually for the duration of time that participants are having disease response evaluations per protocol. Ad hoc meetings may be called at more frequent intervals for cause at the request of the PI or the independent reviewers.

During these meetings, the committee will review the results of the findings from the procedures described in Section **6.3.5** Validation of Disease Assessment (Safety Mechanisms) to ensure disease assessment is managed accurately in accordance with protocol requirements. These results will be provided to the IMC by the PI or designee.

The PI will be responsible for promptly alerting the independent reviewers of any missed disease assessments identified during the validation process. Event reporting and event management will be submitted for appropriate adjudication and reported promptly to the IRB.

8 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATOR

Principal Investigator shall report any SAE, SUSAR, Medical Device Event, Device Deficiency or Incident_information, including, but not limited to, all initial and follow-up information involving any Study subject in the Study.

Serious Adverse Event and Suspected Unexpected Serious Adverse Reaction, Medical Device Event, Potential Incident, Device Deficiency or Incident Reporting: Principal Investigator shall report any SAE, SUSAR, Medical Device Event, Device Deficiency or Incident information, including, but not limited to, all initial and follow-up information involving any Study subject in the Study. Notification shall be in the form of a completed CIOMS I/MedWatch (or other mutually agreed upon format) within two (2) business days of learning of the information. This information shall be transmitted to Merck GS using the contact information provided below or such other modified contact information as provided by Merck in writing. All information shall be transmitted in the English language and contain the reporter's name and the Study subject identifier code. SUSAR information will be reported unblinded if the Study Drug has been blinded in the Study. Randomization codes for all other SAEs will be provided to Merck GS at end of Study if the Study Drug has been blinded in the Study.

Merck may define certain Non-Serious Events of Interest. If any Non-Serious Events of Interest are defined, Merck will provide such information in writing to Principal Investigator at the time of Protocol approval, execution of the CTA or anytime thereafter. Reporting of any defined Non-Serious Events of Interest will be handled in the same manner as SAEs unless mutually agreed otherwise in writing by the parties.

All reports of Study Drug exposure during pregnancy or lactation (including a female partner of a male Study subject using the Study Drug), whether associated with an AE or not, must be reported to Merck GS in accordance with the timelines and contact information for an SAE. Principal Investigator shall follow pregnancies to term to obtain the outcome of the pregnancy. The outcome of the pregnancy shall be forwarded to Merck GS.

The Principal Investigator shall fully comply with all of their respective reporting obligations to the applicable regulatory authorities with respect to any AE, SAE or SUSAR that arises from the Study.

SAE reports and any other relevant safety information are to be forwarded to Merck GS facsimile number: 215-993-1220.

A MedWatch form available at http://www.fda.gov/medwatch/

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary efficacy endpoints:

Safety: Determination of the MTD assessed at the completion of cycle 1 (21 days)

Efficacy: Freedom from distant new brain metastases following stereotactic radiosurgery or surgical resection at 1 year, measured by rate of brain relapse free survival (RFS) for temozolomide and TDM-1 vs. TDM-1 alone assessed at approximately 12-24 months after the last participant starts treatment.

Secondary Efficacy endpoints:

Number of participants with DLTs at each dose level assessed at the completion of cycle 1 (21 days)

Tabulated counts of adverse events assessed through 30 days after the completion of study therapy

Time to whole brain irradiation compared between the two arms assessed at time of whole brain irradiation

Overall survival compared between the two arms assessed at death

9.2 SAMPLE SIZE DETERMINATION

Phase I:

The initial safety evaluation will be done using a standard 3+3 design. Thus, with 3 dose levels, up to 18 participants may be included in the initial safety evaluation.

Phase II:

Following a safety evaluation, the primary objective of the trial is to determine if the addition of temozolomide to TDM-1 will be associated with an increase in new brain metastases Relapse Free Survival (brain RFS) among participants with breast cancer and resection of brain metastases.

While there are no published reports on participants with HER2+ disease and SRS without WBRT, one paper indicated that the 12 month brain RFS is approximately 51% in participants with breast cancer and resected brain metastases that are treated with SRS alone [117]. This will be used to help estimate the sample size. The goal would be to determine if this can be increased by a modest amount with the addition of temozolomide. T-DM1 will be used as the backbone systemic treatment. Initially, a small (3 dose level) phase I-type run-in for safety will be needed for evaluation of T-DM1 and temozolomide combination.

In the main, randomized cohort of the trial, Kaplan-Meier curves and a one-tailed log-rank test will be the primary analysis methods. Assuming exponential brain RFS curves, the hazard rate for TDM-1 associated with a 50% 12 month brain RFS is 0.0578: approximately a 5.8% probability of relapsing each month when the 12 month brain RFS probability is 50%. If we assume that the addition of temozolomide will increase brain RFS to 65% at 12 months, this corresponds to a hazard rate of 0.0359 and the resulting hazard ratio for the comparison of the two overall actuarial curves would be 1.61. Following the principles of a phase 2.5 trial, to compare these curves and detect a difference with a 0.10 significance level one-tailed log-rank test, a total of 49 evaluable subjects per arm (98 total) will need to be randomized over a 3-year period and followed for an additional 2 years from the date of entry of the last participant, with occurrence of 79 total relapses in both arms combined, to have 80% power to compare the curves. It should also be noted that if the true 12 month brain RFS probability without temozolomide were closer to 55%, that 49 participants per arm would provide 83% power for a comparison with 70%, using otherwise identical parameters.

Participants will be randomized to receive TDM-1 or TDM-1 + temozolomide after being registered onto the study, and will be stratified prior to randomization for characteristics potentially associated with relapse: 1 brain lesion in the absence of extracranial disease or presence of stable extracranial disease, 2-5 brain lesions in the absence of extracranial disease or stable extracranial disease, >5 brain lesions in the absence of extracranial disease or stable extracranial disease, 1 brain lesion and progressive extracranial disease, 2-5 brain lesions and progressive extracranial disease, >5 brain lesions and progressive extracranial disease. If participants develop additional systemic lesions and require new therapy, they will be taken off treatment. This group will be followed separately to determine their brain RFS. Additional participants will be accrued to meet the 49 goal with one year of follow up.

It is expected that approximately 30 to 40 participants per year can be accrued onto this trial, and thus accrual to both phases of the trial will be completed in approximately 3-4 years. Allowing for a very small number of inevaluable participants, the accrual ceiling will be set at 125 participants to permit up to 18 participants in the initial dose escalation phase and 98 randomized evaluable participants.

9.3 POPULATIONS FOR ANALYSIS

Phase I: all participants who received at least one dose of the agents

Phase II: Modified intention to treat analysis (all participants who received at least one dose of the agents)

9.4 STATISTICAL ANALYSES

9.4.1 General approach

Safety data will be tabulated and reported per arm.

Kaplan-Meier curves will be created for each of the randomized arms and compared using a two-tailed log-rank test, with a one-sided 0.10 significance level of interest to be detected.

9.4.2 Analysis of the primary safety and efficacy endpoints

The MTD will be identified based on being the dose level at which 0 or 1 participant in 6 has a DLT.

Brain RFS curves will be created using the Kaplan-Meier method based on all participants considered to be evaluable based on having received protocol treatment, and compared using a one-tailed log-rank test, with a one-sided 0.10 significance level of interest to be detected.

9.4.3 Analysis of the secondary efficacy endpoints

Number of participants with DLTs at each dose level: tabulated counts

Toxicity data: tabulated counts

Time to whole brain irradiation compared between the two arms: Kaplan-Meier curves constructed with a two-tailed log-rank test used to compare the arms.

Overall survival compared between the two arms Kaplan-Meier curves constructed with a two-tailed log-rank test used to compare the arms.

9.4.4 Safety Analyses

Toxicity data: tabulated counts and adverse events will be evaluated descriptively.

9.4.5 Baseline Descriptive Statistics

Demographic and clinical characteristics of participants on both arms will be tabulated.

9.4.6 Planned interim analyses

This study will be monitored by the NCI/CCR Data Safety and Monitoring Board, both for toxicity as well as futility and efficacy with respect to the primary brain RFS endpoint.

Beginning in the year after twenty total participants have been randomized and treated on the trial, the study will be monitored by a DSMB on an annual basis to evaluate the safety of the two arms. The SAEs (typically grade 3 toxicities, or greater) will be reported according to type of toxicity, and maximal grade noted per participant, for toxicities with at least a possible attribution to the therapy provided on that arm. Comparisons will be made between the two arms using a Cochran-Armitage test for trend, or other appropriate methods, to determine if there is increased toxicity associated with either arm.

In addition, at the first DSMB meeting held following the point at which half of the required total subjects have been enrolled and followed for a minimum of 12 months (that is, after 49 participants have been enrolled, and the most recent of those 49 participants were randomized at least 12 months prior to the date of the DSMB evaluation), a single evaluation for futility will be undertaken. The futility evaluation will be performed as follows: based on the full data available at the time, if the hazard ratio for brain RFS for the two arms is not favoring the temozolomide

arm (that is, if the HR=1.0 or greater favoring TDM-1 alone), or at the discretion of the DSMB if results are not clearly defined for other reasons, such as the appearance of the interim Kaplan-Meier curves, then the trial will end accrual at that time if this is directed by the DSMB.

Monitoring for efficacy will take place on an annual basis, also beginning after 49 participants have been randomized and followed potentially for a minimum of 6 months. This will be performed using a Lan-DeMets alpha spending function approach based on an O'Brien-Fleming boundary associated with the results of the log-rank test related to the comparison of OS curves.

9.4.7 Exploratory analyses

As described in the correlative studies section of the protocol, biomarkers in the CSF and serum will be measured, as well as their changes with treatment. The biomarkers will be evaluated in an exploratory fashion to predict relapse.

MGMT methylation status will be analyzed in primary tumor, CSF and serum, as described in the correlative studies section in the protocol. Findings at baseline and changes at different points during the treatment will be compared between participants that benefit and those participants that don't benefit from temozolomide treatment, applying non-parametric tests.

In the same manner, questionnaires regarding cognitive and quality of life aspects will be applied and analyzed in an exploratory fashion. The analysis should include:

1. Neurotoxicity and neurocognitive effects between the two arms

2. MDASI-BT subscale (cognitive dysfunction, focal neurologic deficits, and interference) differences between the two arms

3. Baseline MDASI-BT subscales (overall symptom burden, overall interference, cognitive dysfunction) as well as fatigue and change of appetite as prognostic for time to distant metastases and for OS

4. Changes in score over time in select MDASI-BT subscales as prognostic for OS, time to distant metastases, and neurocognitive decline

10 COLLABORATIVE AGREEMENTS

10.1 CLINICAL TRIALS AGREEMENT (CTA)

The study agent, Temozolomide is provided by MERCK & Co., Inc. under a Clinical Trials Agreement (CTA). Results or study data may be communicated to CTA partner, according to the terms of the NIH- Advanced Accelerator Applications SA CTA.

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial and ethnic groups are eligible for this trial if they meet the eligibility criteria. Efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to racial or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

We are excluding non-English speakers because part of our study objectives is to evaluate neurocognitive effects of disease and treatment. The evaluation of neurocognitive effects relies in tests and questionnaires that require clear comprehension of the language used to apply them. Failures in comprehension of the language could jeopardize the tests results and interpretation.

11.2 PARTICIPATION OF CHILDREN

The age group for enrollment on this trial is 18 or more years of age. Because no dosing or adverse event data are currently available on the use of T-DM1 or temozolomide in participants < 18 years of age, children are excluded from this study.

11.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **11.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see section **11.6.1** for consent procedure.

11.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The primary risk to participants participating in this research study is from the toxicity of Temozolomide and T-DM1, or both drugs. Temozolomide is an investigational agent in the prevention of breast cancer brain metastases. T-DM1 is an approved agent for treatment of metastatic HER2-positive breast cancer. The protocol provides for detailed and careful monitoring of all participants to assess for toxicity. Toxicity data from the phases I and II dose levels will be collected and reviewed to ensure that there were no severe toxicities that would preclude further participant enrollment. Participants will be treated with therapeutic intent and response to the therapy will be closely monitored.

11.5 RISKS/BENEFITS ANALYSIS

11.5.1 Risks

11.5.2 Research Blood Collection Risks

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

11.5.3 Risks of Echocardiogram

Other than the possibility of some mild discomfort during the test, there are no known risks to an echocardiogram.

11.5.4 Risks of MUGA Scans

In addition to the risks of radiation exposure, the primary risk associated with MUGA scans is an allergic reaction to the contrast material.

11.5.5 Risks of Questionnaires

These contain questions that may be sensitive in nature. Subjects are instructed that they may refuse to answer any question that makes them feel uncomfortable, and theat if they have concerns after completing the questionnaire, they are encouraged to contact the study team.

11.5.6 Lumbar Puncture for Research Purposes

Risks of lumbar puncture include headache, tenderness or pain in the lower back and bleeding near the puncture site; and pain and numbness that shoots down in the legs. In rare cases, people experience brainstem herniation, which is the movement of brain tissue from its normal position in the skull.

11.5.7 Risks of exposure to ionizing radiation

Study participants will be exposed to radiation from the following sources within a one-year period:

- Up to 5 lumbar punctures guided by fluoroscopy
- Up to 8 CT scans
- 1 technetium bone scan
- Up to 4 MUGA scans

The amount of radiation exposure from these procedures is equal to approximately 11.19 rem. This is equal to roughly the same amount of radiation as 37.3 years' worth of background radiation. About 40 out of 100 people (40%) will get cancer during their lifetime, and 20 out of 100 (20%) will die from cancer. The risk of getting cancer from the radiation exposure in this study is 1.1 out of 100 (1.1%) and of getting a fatal cancer is 0.6 out of 100 (0.6%).

Note: MRIs will be performed for participants who are unable to tolerate the CT contrast.

11.5.8 Risks of CT Scans

In addition to the radiation risks discussed above, risks associated with CT scans include allergic reaction to and kidney damage from the contrast dye, nausea, vomiting, and anxiety.

11.5.9 Risks of MRIs

The risks of MRI include anxiety as well as the risks associated with the gadolinium contrast material, which are metallic taste, headache, nausea, and allergic reaction; and in participants with kidney disease, nephrogenic systemic fibrosis (NSF).

11.5.10 Other risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

11.5.11 Non-Physical Risks of Genetic Research

11.5.11.1 Risk of receiving unwanted information

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Participants will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with participants, family members or health care providers.

11.5.11.2 Risk related to possibility that information may be released

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the participants, family members or health care providers, this risk will be included in the informed consent document.

11.5.11.3 Risk to family or relatives

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems. As previously noted, participants will be given the option in the consent document to be notified of any medically significant and actionable incidental findings.

11.5.12 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study has a Certificate of Confidentiality which helps to prevent forced disclosure of personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation. The informed consent includes the appropriate coverage and restrictions of the Certificate of Confidentiality.

11.5.13 Benefits

The potential benefit to a participant on this study is a reduction or stability in the volume of their tumor systemically or outside of the brain, as well as potentially the prevention or delay in development of new metastatic lesions in the brain, which may or may not have favorable impact on symptoms and/or survival.

11.5.14 Risks/Benefits Analysis

The potential benefits from this therapy are stabilization or shrinkage of disease outside the brain and a reduction in chances of developing new tumor lesions in the brain, with decrease of symptoms caused by the brain tumor such as neurological deficits and headache. Given the efforts to minimize risk with the administration of this combination, this protocol involves greater than minimal risk, but presents the potential for direct benefit to individual subjects.

11.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time

as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the subject will sign the document using a finger, stylus or mouse.

> Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

11.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **11.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **11.6**.

11.7 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with

additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

There will be no IND obtained for the use of any of the commercial agents used in this study.

This study meets the criteria for exemption for an IND as this investigation is not intended to support a new indication for use or any other significant change to the labeling; the drugs are already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

13.1 TEMOZOLOMIDE

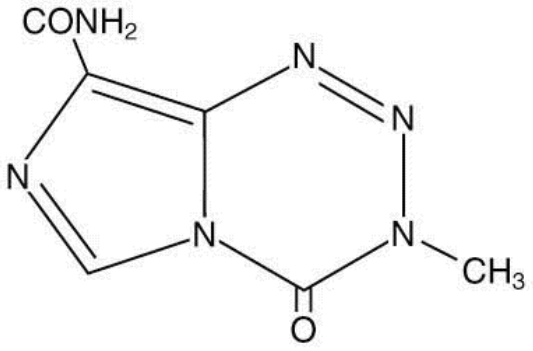
13.1.1 Source

Temozolomide is commercially available. It will be provided by MERCK & Co., Inc. for this study under the agreement described in section **10.1**.

NOTE: Effective with Amendment F (version date 06/08/2020): Merck discontinuned production of the Temozolomide 5mg and 20mg capsules, so Merck will purchase those doses from Myonex for delivery to the NIH pharmacy. Merck will continue to provide the 100mg capsules per the original agreement.

13.1.2 Formulation and preparation

Temozolomide is an imidazotetrazine derivative. The chemical name of temozolomide is 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide. The structural formula is:



The material is a white to light tan/light pink powder with a molecular formula of C6H6N6O2 and a molecular weight of 194.15. The molecule is stable at acidic pH (<5) and labile at pH >7; hence Temozolomide can be administered orally and intravenously. The prodrug, temozolomide, is rapidly hydrolyzed to the active 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) at neutral andalkaline pH values, with hydrolysis taking place even faster at alkaline pH.

Other Names: methazolastone, Temodar

Temozolomide is supplied in white opaque, preservative-free, two-piece, hard gelatin capsules of the following p.o. dosage strengths: 5 mg, 20 mg, 100 mg, 180 mg and 250 mg. Each capsule contains drug substance in combination with lactose, anhydrous NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid NF, and stearic acid NF. The capsule shells contain gelatin NF, titanium dioxide USP, and sodium lautyl sulfate NF.

Mode of Action

Alkylating agent of imidazotetrazinone class.

13.1.3 Storage and Stability

The 5-mg, 20-mg, 100-mg, 140-mg, and 180-mg capsule strengths are available in 5- count and 14-count packages. The 250-mg capsule strength is available in a 5-count package. Each strength of Temozolomide must be dispensed in a separate vial or in its original package (one strength per one container).

Follow the instructions below:

Based on the dose prescribed, determine the number of each strength of Temozolomide capsules that are needed. Label each container with the appropriate number of capsules to be taken each day. Dispense to the participant, making sure each container lists the strength (mg) per capsule and that he or she understands to take the appropriate number of capsules of Temozolomide from each package or vial to equal the total daily dose prescribed by the physician. Temozolomide is store at 25°C, excursions permitted to 15-30°C.

Repackaging of Temozolomide for dispensing may occur per guidance provided in manufacturer's product labeling.

13.1.4 Administration procedures

Participants should take each day’s dose with a full glass of water at the same time each day. Taking the medication on an empty stomach or at bedtime may help ease nausea. If participants are also taking anti-nausea or other medications to relieve the side effects associated with Temozolomide, they should be advised to take these medications 30 minutes before they take Temozolomide. Temozolomide causes the rapid appearance of malignant tumors in rats. Participants SHOULD NOT open or split the capsules. If capsules are accidentally opened or damaged, rigorous precautions should be taken with the capsule contents to avoid inhalation or contact with the skin or mucous membranes. The medication should be kept away from children and pets. The Temozolomide capsules should be swallowed whole and NEVER CHEWED.

13.1.5 Pharmacokinetics

Temozolomide is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in 1 hour. Food reduces the rate and extent of temozolomide absorption. Mean peak plasma concentration and AUC decreased by 32% and 9%, respectively, and Tmax increased 2-fold (from 1.1 to 2.25 hours) when temozolomide was administered after a modified high-fat breakfast. Temozolomide is rapidly eliminated with a mean elimination half-life of 1.8 hours and exhibits linear kinetics over the therapeutic dosing range. Temozolomide has a mean apparent volume of distribution of 0.4 L/kg (%CV=13%). It is weakly bound to human plasma proteins; the mean percent bound of drug-related total radioactivity is 15%.

13.1.6 Metabolism and Elimination

Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species, 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC) and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the

metabolism of temozolomide and MTIC. Relative to the AUC of temozolomide, the exposure to MTIC and ACI is 2.4% and 23%, respectively. About 38% of the administered temozolomide total radioactive dose is recovered over 7 days; 37.7% in urine and 0.8% in feces. The majority of the recovery of radioactivity in urine is as unchanged temozolomide (5.6%), AIC (12%), temozolomide acid metabolite (2.3%), and unidentified polar metabolites(s) (17%). Overall clearance of temozolomide is about 5.5 L/hr/m^2.

13.1.7 Special Populations

Creatinine Clearance: Population pharmacokinetic analysis indicates that creatinine clearance over the range of 36-130 mL/min/m2 has no effect on the clearance of temozolomide after oral administration. The pharmacokinetics of temozolomide have not been studied in participants with severely impaired renal function (CLcr < 36 mL/min/m^2). Caution should be exercised when temozolomide is administered to participants with severe renal impairment. Temozolomide has not been studied in participants on dialysis.

Hepatically Impaired Participants: In a pharmacokinetic study, the pharmacokinetics of temozolomide in participants with mild to moderate hepatic impairment (Child's-Pugh Class I-II) were similar to those observed in participants with normal hepatic function. Caution should be exercised when temozolomide is administered to participants with severe hepatic impairment.

Gender: Population pharmacokinetic analysis indicates that women have an approximately 5% lower clearance (adjusted for body surface area) for temozolomide than men. Women have higher incidences of grade 4 neutropenia and thrombocytopenia in the first cycle of therapy than men.

Population pharmacokinetic analysis indicates that age (range 19-78 years) has no influence on the pharmacokinetics of temozolomide. In the anaplastic astrocytoma study population, participants 70 years of age or older had a higher incidence of grade 4 neutropenia and grade 4 thrombocytopenia in the first cycle of therapy than participants under 70 years of age. In the entire safety database, however, there did not appear to be a higher incidence in participants 70 years of age or older.

13.1.8 Incompatibilities and Toxicity

Drug-Drug Interactions: In a multiple dose study, administration of temozolomide with ranitidine did not change the Cmax or AUC values for temozolomide or MTIC. Population analysis indicates that administration of valproic acid decreases the clearance of temozolomide by about 5%. The clinical implication of this effect is not known. Population analysis failed to demonstrate any influence of co-administered dexamethasone, prochlorperazine, phenytoin, carbamazepine, ondansetron, H2- receptor antagonists, or phenobarbital on the clearance of orally administered temozolomide.

Known Potential Adverse Events

Hematologic: Thrombocytopenia, leukopenia, myelodysplastic syndrome

Gastrointestinal: Nausea, vomiting, anorexia

Hepatic: Elevated liver enzymes (reversible)

Skin: Rash

Neurologic: Convulsions, weakness on one side of the body, abnormal coordination, paralysis

Other: Constipation, diarrhea, stomatitis, fatigue, decreased performance status, headache

Likely (occurring in more than 20% of participants)

Fatigue	Alopecia	Constipation
Headache	Nausea	Anorexia
Seizures	Vomiting	

Common (occurring in 3-20% of participants)

Dizziness	Depression	Weight gain
Abnormal muscle movements/ coordination	Difficulty sleeping	Loss of urinary control
Abnormal gait	Drowsiness	Urinary tract infection
Hemiplegia or partial paralysis	Skin rash	Frequent urination
Upper and/or lower extremity edema	Pruritus	Abnormal vision
Weakness (such as weakness on one side of the body)	Dry skin	Blurry vision
Tickling/tingling sensation	Mucositis	Diplopia or double vision
Pain (such as in the abdomen, joints, back, and/or muscles)	Dysphagia	Fever
Breast pain in females	Dysgeusia or taste changes	Head cold
Confusion	Diarrhea	Cough
Memory problems	Excess steroid in the body (possible bruising and/or increase in size of the face and/or neck)	Sore throat
Anxiety	Viral infection	Sinusitis
Dyspnea	Allergic reaction	

Temozolomide may commonly cause low blood cell counts (white blood cells, red blood cells, and platelets). This means that while you take the drug, there is more of a chance of getting an infection, including pneumonia. You may become anemic and/or have problems with bleeding, bruising, fatigue, and/or shortness of breath. You may need a blood transfusion.

Rare but serious (occurring in fewer than 3% of participants)

Bone marrow disease where not enough blood cells are made	New occurrence of cancer (including myeloid leukemia)	Steven-Johnson Syndrome
Hallucinations	Allergic skin reaction	Damage from radiation (such as skin damage)
Nervous system disease (possible pain and/or weakness)	Severe skin damage with loss of a large portion of skin	Pneumonitis (lung inflammation)
Neuropathies (nerve damage- possible numbness, tingling, and pain)	Weight loss	Flu-like symptoms
Hyperglycemia (possible diabetes)	Fever due to low white blood cell counts	Injection site reactions (skin redness, irritation, pain, itching, swelling, and/or warmth)

Hypokalemia (possible weakness)	Bruising	Opportunistic infection
Severe allergic reaction	Hemorrhage	Herpes infection causing painful skin rash (shingles)

The following side effects have been reported in research studies with temozolomide. It is unclear if these side effects were caused by temozolomide, but they may be:

BUN blood level increase	Death	Cholecystitis
Creatinine blood elevated	Hypoxia	Pancreatitis
Lymphopenia	Weight loss	GI perforation
Thrombocytopenia	Epistaxis	Dehydration
Neutropenia	Sepsis	

Temozolomide is potentially mutagenic and should be handled with appropriate precautions by both staff and participants. Capsules should not be opened. If capsules are accidentally opened or damaged, rigorous precautions should be taken with the capsule contents to avoid inhalation or contact with the skin or mucous membranes. Procedures for proper handling and disposal of anticancer drugs should be considered.

Contraindications: Temozolomide is contraindicated in participants who have a history of a hypersensitivity reaction to any of its components or to DTIC.

Pregnancy Category D

Temozolomide may cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women. If this drug is used during pregnancy, or if the participant becomes pregnant while taking this drug, the participant should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant during therapy with temozolomide.

Treatment of a man with temozolomide may increase the risk of birth defects if he causes a woman to become pregnant while he is taking temozolomide. Men treated with temozolomide may have difficulty causing a woman to become pregnant after their treatment is completed. Men receiving temozolomide should be directed to use effective contraception while they are being treated. There is insufficient data to know what the risk of subsequent problems with fertility will be. Similarly, women who are treated with temozolomide may have difficulty becoming pregnant in the future and may at be at increased risk of having children with birth defects. There is insufficient evidence to determine what the risk of these complications will be.

13.2 T-DM1

13.2.1 Source

T-DM1 will be used in its standard approved indication by the FDA.

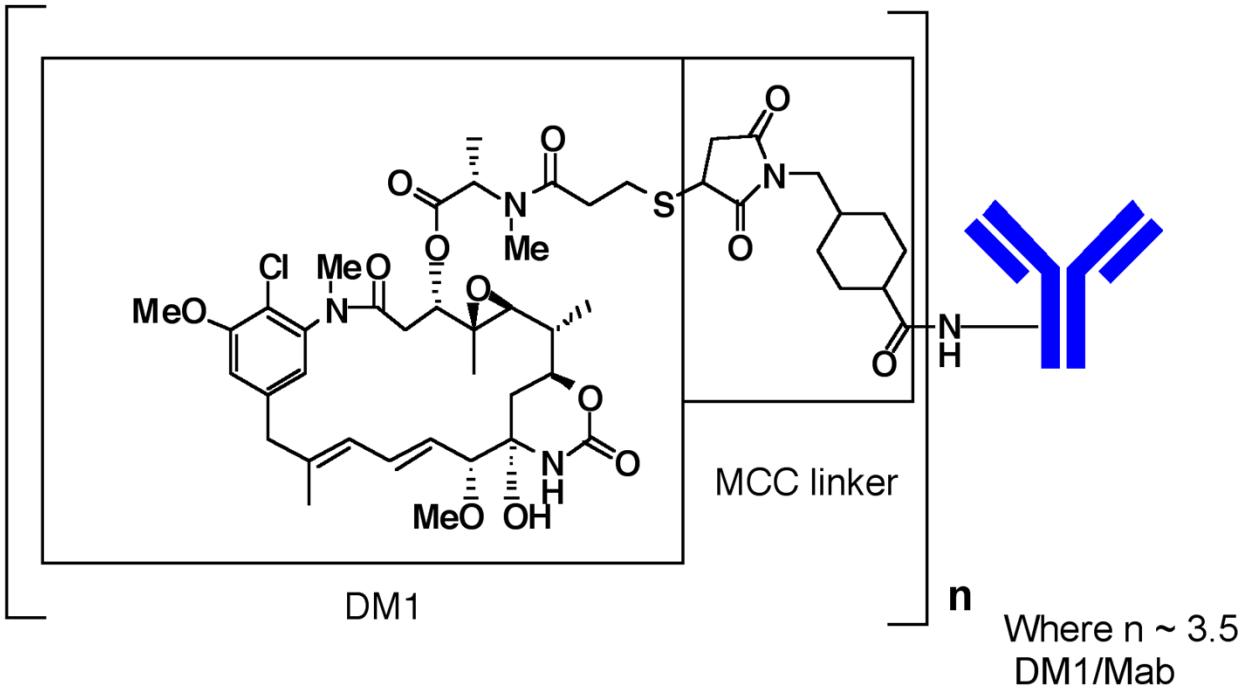
T-DM1 will be purchased from commercial sources by the NIH CC Pharmacy.

13.2.2 Formulation and Preparation

T-DM1 (ado-trastuzumab emtansine) is a HER2-targeted antibody-drug conjugate (ADC) which contains the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule

inhibitory drug DM1 (a maytansine derivative) via the stable thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate). Emtansine refers to the MCC-DM1 complex.

The antibody trastuzumab, is a well characterized recombinant monoclonal antibody product produced by mammalian (Chinese hamster ovary) cells, and the small molecule components (DM1 and MCC) are produced by chemical synthesis. Ado-trastuzumab emtansine contains an average of 3.5 DM1 molecules per antibody. Ado-trastuzumab emtansine has the following chemical structure:



Note: The bracketed structure is DM1 plus MCC which represents the emtansine component. The n is, on average, 3.5 DM1 molecules per trastuzumab (Mab) molecule.

T-DM1 (ado-trastuzumab emtansine) is a sterile, white to off-white preservative free lyophilized powder in single-use vials. Each vial contains 100 mg or 160 mg ado-trastuzumab emtansine. Following reconstitution, each single-use vial contains ado-trastuzumab emtansine (20 mg/mL), polysorbate 20 [0.02% (w/v)], sodium succinate (10 mM), and sucrose [6% (w/v)] with a pH of 5.0 and density of 1.026 g/mL. The resulting solution containing 20 mg/mL ado-trastuzumab emtansine is administered by intravenous infusion following dilution.

13.2.3 Supply and handling:

T-DM1(ado-trastuzumab emtansine) is supplied as: one 100 mg vial, single use vial or one 160 mg vial, single use vial per carton.

Store vials in a refrigerator at 2°C to 8°C (36°F to 46°F) until time of reconstitution. *Do not freeze or shake.*

13.2.4 Formulation and preparation

In order to prevent medication errors, it is important to check the vial labels to ensure that the drug being prepared and administered isT-DM1 (ado-trastuzumab emtansine) and not trastuzumab. Lyophilized powder in single-use vials: 100 mg per vial or 160 mg per vial of ado-trastuzumab emtansine.

13.2.5 Administration

Administer T-DM1 as an intravenous infusion only with a 0.2 or 0.22 micron in-line polyethersulfone (PES) filter. Do not administer as an intravenous push or bolus. Do not mix T-DM1, or administer as an infusion, with other medicinal products. In order to improve traceability of biological medicinal products, the tradename and the batch number of the administered product should be clearly recorded (or stated) in the patient file.

13.2.5.1 Reconstitution

Use aseptic technique for reconstitution and preparation of dosing solution. Appropriate procedures for the preparation of chemotherapeutic drugs should be used.

Using a sterile syringe, slowly inject 5 mL of Sterile Water for Injection into the 100 mg T-DM1 vial, or 8 mL of Sterile Water for Injection into the 160 mg T-DM1 vial to yield a solution containing 20 mg/mL. Swirl the vial gently until completely dissolved. *Do not shake.* Inspect the reconstituted solution for particulates and discoloration.

The reconstituted solution should be clear to slightly opalescent and free of visible particulates. The color of the reconstituted solution should be colorless to pale brown. Do not use if the reconstituted solution contains visible particulates or is cloudy or discolored.

The reconstituted lyophilized vials should be used immediately following reconstitution with Sterile Water for Injection. If not used immediately, the reconstituted T-DM1 vials can be stored for up to 24 hours in a refrigerator at 2ºC to 8ºC (36°F to 46°F); discard unused T-DM1 after 24 hours. *Do not freeze.*

The reconstituted product contains no preservative and is intended for single-use only.

13.2.5.2 Dilution

Determine the correct dose (mg) of T-DM1.

Calculate the volume of the 20 mg/mL reconstituted T-DM1 solution needed.

Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% Sodium Chloride Injection. *Do not use Dextrose (5%) solution.*

Gently invert the bag to mix the solution in order to avoid foaming.

The diluted T-DM1 infusion solution should be used immediately. If not used immediately, the solution may be stored in a refrigerator at 2°C to 8°C (36°F to 46°F) for up to 24 hours prior to use. This storage time is additional to the time allowed for the reconstituted vials. *Do not freeze or shake.*

13.2.6 Pharmacokinetics

The pharmacokinetics of T-DM1 was evaluated in a phase 1 study and in a population pharmacokinetic analysis for the ado-trastuzumab emtansine conjugate (ADC) using pooled data

from 5 trials in participants with breast cancer. A linear two-compartment model with first-order elimination from the central compartment adequately describes the ADC concentration-time profile. In addition to ADC, the pharmacokinetics of total antibody (conjugated and unconjugated trastuzumab), DM1 were also determined. The pharmacokinetics of T-DM1 are summarized below.

13.2.7 Distribution

Maximum concentrations (Cmax) of ADC and DM1 were observed close to the end of infusion. In Study 1, mean (SD) ADC and DM1 Cycle 1 Cmax following KADCYLA administration was 83.4 (16.5) µg/mL and 4.61 (1.61) ng/mL, respectively. 18 of 24

In vitro, the mean binding of DM1 to human plasma proteins was 93%. *In vitro,* DM1 was a substrate of P-glycoprotein (P-gp). Based on population pharmacokinetic analysis, the central volume of distribution of ADC was 3.13 L.

13.2.8 Metabolism and Elimination

In vitro studies indicate that DM1, the small molecule component of T-DM1, undergoes metabolism by CYP3A4/5. DM1 did not inhibit or induce major CYP450 enzymes *in vitro.* In human plasma, ado-trastuzumab emtansine catabolites MCC-DM1, Lys-MCC-DM1, and DM1 were detected at low levels.

Based on population pharmacokinetic analysis, following intravenous infusion of T-DM1, the clearance of the ADC was 0.68 L/day and the elimination half-life (t1/2) was approximately 4 days. No accumulation of T-DM1 was observed after repeated dosing of intravenous infusion every 3 weeks.

Based on population pharmacokinetic analysis (n=671), body weight, sum of longest diameter of target lesions by RECIST, HER2 extracellular domain (ECD) concentrations, AST, albumin, and baseline trastuzumab concentrations were identified as statistically significant covariates for ado-trastuzumab emtansine clearance. However, the magnitude of effect of these covariates on ado-trastuzumab emtansine exposure suggests that, with the exception of body weight, these covariates are unlikely to have a clinically meaningful effect on T-DM1 exposure. Therefore, the body weight based dose of 3.6 mg/kg every 3 weeks without correction for other covariates is considered appropriate.

Drug to drug interaction: No formal drug-drug interaction studies with T-DM1 have been conducted. *In vitro* studies indicate that DM1, the cytotoxic component of T-DM1, is metabolized mainly by CYP3A4 and to a lesser extent by CYP3A5. Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) with T-DM1 should be avoided due to the potential for an increase in DM1 exposure and toxicity. Consider an alternate medication with no or minimal potential to inhibit CYP3A4. If concomitant use of strong CYP3A4 inhibitors is unavoidable, consider delaying T-DM1 treatment until the strong CYP3A4 inhibitors have cleared from the circulation (approximately 3 elimination half-lives of the inhibitors) when possible. If a strong CYP3A4 inhibitor is coadministered and T-DM1 treatment cannot be delayed, participants should be closely monitored for adverse reactions.

13.2.9 Special populations

Pregnancy:

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to T-DM1 during pregnancy. Encourage women who receive T-DM1 during pregnancy or within 7 months prior to conception, to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 or visiting http://www.motherpregnancyregistry.com/.

In addition, there is a pregnancy pharmacovigilance program for T-DM1. If T-DM1 is administered during pregnancy, or if a participant becomes pregnant while receiving T-DM1 or within 7 months following the last dose of T-DM1, health care providers and participants should immediately report T-DM1 exposure to Genentech at 1-888-835-2555.

T-DM1 can cause fetal harm when administered to a pregnant woman. There are no available data on the use of T-DM1 in pregnant women. Cases of oligohydramnios and oligohydramnios sequence manifesting as pulmonary hypoplasia, skeletal abnormalities, and neonatal death were observed in the postmarketing setting in participants treated with trastuzumab, the antibody component of T-DM1. Based on its mechanism of action, the DM1 component of T-DM1 can also cause embryo-fetal harm when administered to a pregnant woman. Apprise the participant of the potential risks to a fetus. There are clinical considerations if T-DM1 is used in a pregnant woman, or if a participant becomes pregnant within 7 months following the last dose of T-DM1.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Fetal/Neonatal Adverse Reactions Monitor women who received T-DM1 during pregnancy or within 7 months prior to conception for oligohydramnios. If oligohydramnios occurs, perform fetal testing that is appropriate for gestational age and consistent with community standards of care.

Human Data There are no available data on the use of T-DM1 in pregnant women. In the post-marketing setting, cases of oligohydramnios, and of oligohydramnios sequence, manifesting in the fetus as pulmonary hypoplasia, skeletal abnormalities and neonatal death were observed after treatment with trastuzumab during pregnancy. These case reports described oligohydramnios in pregnant women who received trastuzumab either alone or in combination with chemotherapy. In some case reports, amniotic fluid index increased after trastuzumab was stopped. In one case, trastuzumab therapy resumed after amniotic index improved, and oligohydramnios recurred.

Animal Data There were no reproductive and developmental toxicology studies conducted with ado-trastuzumab emtansine. DM1, the cytotoxic component of T-DM1, disrupts microtubule function. DM1 is toxic to rapidly dividing cells in animals and is genotoxic, suggesting it has the potential to cause embryotoxicity and teratogenicity. In studies where trastuzumab was administered to pregnant cynomolgus monkeys during the period of organogenesis at doses up to 25 mg/kg given twice weekly (about 7 times the clinical dose), trastuzumab crossed the placental barrier during the early (Gestation Days 20 to 50) and late (Gestation Days 120 to 150) phases of gestation. The resulting concentrations of trastuzumab in fetal serum and amniotic fluid were 15 of 24 approximately 33% and 25%, respectively, of those present in the maternal serum but were not associated with adverse developmental effects.

Lactation

There is no information regarding the presence of ado-trastuzumab emtansine in human milk, the effects on the breastfed infant, or the effects on milk production. DM1, the cytotoxic component of T-DM1, may cause serious adverse reactions in breastfed infants based on its mechanism of

action. Advise women not to breastfeed during treatment and for 7 months following the last dose of T-DM1.

There were no animal lactation studies conducted with ado-trastuzumab emtansine or the cytotoxic component of T-DM1 (DM1). In lactating cynomolgus monkeys, trastuzumab was present in breast milk at about 0.3% of maternal serum concentrations after pre- (beginning Gestation Day 120) and post-partum (through Post-partum Day 28) doses of 25 mg/kg administered twice weekly (about 7 times the clinical dose of T-DM1). Infant monkeys with detectable serum levels of trastuzumab did not exhibit any adverse effects on growth or development from birth to 1 month of age.

Verify the pregnancy status of females of reproductive potential prior to the initiation of T-DM1.

Contraception

Females T-DM1 can cause embryo-fetal harm when administered during pregnancy. Advise females of reproductive potential to use effective contraception during treatment and for 7 months following the last dose of T-DM1

Males Because of the potential for genotoxicity, advise male participants with female partners of reproductive potential to use effective contraception during treatment with T-DM1 and for 4 months following the last dose.

Infertility Based on results from animal toxicity studies, T-DM1 may impair fertility in females and males of reproductive potential. It is not known if the effects are reversible

Geriatric Use

Of 495 participants who were randomized to T-DM1 in the randomized trial, 65 participants (13%) were ≥ 65 years of age and 11 participants (2%) were ≥ 75 years of age. In participants ≥ 65 years old (n=138 across both treatment arms) the hazard ratios for progression-free survival (PFS) and Overall Survival (OS) were 1.06 (95% CI: 0.68, 1.66) and 1.05 (95% CI: 0.58, 1.91), respectively. Population pharmacokinetic analysis indicates that age does not have a clinically meaningful effect on the pharmacokinetics of ado-trastuzumab emtansine

Renal Impairment

No dedicated renal impairment trial for T-DM1 has been conducted. Based on the population pharmacokinetics, as well as analysis of Grade 3 or greater adverse drug reactions and dose modifications, dose adjustments of T-DM1 are not needed in participants with mild (creatinine clearance [CLcr] 60 to 89 mL/min) or moderate (CLcr 30 to 59 mL/min) renal impairment. No dose adjustment can be recommended for participants with severe renal impairment (CLcr less than 30 mL/min) because of the limited data available.

Hepatic Impairment

No adjustment to the starting dose is required for participants with mild or moderate hepatic impairment. T-DM1 was not studied in patients with severe hepatic impairment. Closely monitor participants with hepatic impairment due to known hepatotoxicity observed with T-DM1.

13.2.10 Incompatibilities and Toxicity

Incompatibilities are addressed in Section **4.1.1**

Known Potential Adverse Events

Hematologic: Thrombocytopenia, neutropenia, anemia

Gastrointestinal: Nausea, vomiting, diarrhea

Hepatic: Elevated liver enzymes

Skin: Rash, pruritus

Neurologic: headache, peripheral neuropathy

Other: fatigue, decreased performance status

Likely (occurring in more than 25% of participants)

Fatigue	Hemorrhage	Increased transaminases
Nausea	Thrombocytopenia	Constipation
Musculoskeletal pain	Headache	Epistaxis

Common (occurring in 3-25% of participants)

Blood alkaline phosphatase increased	Dysgeusia	Dyspnea
Hypokalemia	Dizziness	Cough
Myalgia	Insomnia	Pruritus
Arthralgia	Rash	Hypertension
Increased bilirubin		

Rare but serious / G3-4 (occurring in fewer than 3% of participants)

Hypokalemia	Musculoskeletal pain	Peripheral neuropathy
Hemorrhage	Hypertension	

Hepatic failure has been observed in two participants (0.2%) with HER2-positive metastatic breast cancer in clinical trials (n=884) with T-DM1 as single-agent.

14 REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. *Ca-a Cancer Journal for Clinicians* 2015; **65**(1): 5-29.
2. Barnholtz-Sloan JS, Sloan AE, Davis FG, Vigneau FD, Lai P, Sawaya RE. Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the metropolitan Detroit cancer surveillance system. *Journal of Clinical Oncology* 2004; **22**(14): 2865-72.
3. Arvold ND, Oh KS, Niemierko A, et al. Brain metastases after breast-conserving therapy and systemic therapy: incidence and characteristics by biologic subtype. *Breast Cancer Research and Treatment* 2012; **136**(1): 153-60.
4. Kennecke H, Yerushalmi R, Woods R, et al. Metastatic Behavior of Breast Cancer Subtypes. *Journal of Clinical Oncology* 2010; **28**(20): 3271-7.
5. Bendell JC, Domchek SM, Burstein HJ, et al. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer* 2003; **97**(12): 2972-7.
6. Clayton AJ, Danson S, Jolly S, et al. Incidence of cerebral metastases in patients treated with trastuzumab for metastatic breast cancer. *British Journal of Cancer* 2004; **91**(4): 639-43.
7. Stemmler HJ, Kahlert S, Siekiera W, Untch M, Heinrich B, Heinemann V. Characteristics of patients with brain metastases receiving trastuzumab for HER2 overexpressing metastatic breast cancer. *Breast* 2006; **15**(2): 219-25.
8. Yau T, Swanton C, Chua S, et al. Incidence, pattern and timing of brain metastases among patients with advanced breast cancer treated with trastuzumab. *Acta Oncologica* 2006; **45**(2): 196-201.
9. Pestalozzi BC, Brignoli S. Herceptin(R) (trastuzumab) in cerebrospinal fluid (CSF). *European Journal of Cancer* 2000; **36**(Supplement 5): S54-S.
10. Stemmler J, Schmitt M, Willems A, Bernhard H, Harbeck N, Heinemann V. Brain metastases in HER2-overexpressing metastatic breast cancer: Comparative analysis of trastuzumab levels in serum and cerebrospinal fluid. *Journal of Clinical Oncology* 2006; **24**(18): 64S-S.
11. Tsao MN, Rades D, Wirth A, et al. Radiotherapeutic and surgical management for newly diagnosed brain metastasis(es): An American Society for Radiation Oncology evidence-based guideline. *Practical radiation oncology* 2012; **2**(3): 210-25.
12. Andrews DW, Scott CB, Sperduto PW, et al. Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: phase III results of the RTOG 9508 randomised trial. *Lancet* 2004; **363**(9422): 1665-72.
13. Aoyama H, Shirato H, Tago M, et al. Stereotactic radiosurgery plus whole-brain radiation therapy vs stereotactic radiosurgery alone for treatment of brain metastases - A randomized controlled trial. *Jama-Journal of the American Medical Association* 2006; **295**(21): 2483-91.
14. Kocher M, Soffietti R, Abacioglu U, et al. Adjuvant Whole-Brain Radiotherapy Versus Observation After Radiosurgery or Surgical Resection of One to Three Cerebral Metastases: Results of the EORTC 22952-26001 Study. *Journal of Clinical Oncology* 2011; **29**(2): 134-41.
15. Soffietti R, Kocher M, Abacioglu UM, et al. A European Organisation for Research and Treatment of Cancer Phase III Trial of Adjuvant Whole-Brain Radiotherapy Versus Observation in Patients With One to Three Brain Metastases From Solid Tumors After Surgical Resection or Radiosurgery: Quality-of-Life Results. *Journal of Clinical Oncology* 2013; **31**(1): 65-72.

16. Chang EL, Wefel JS, Hess KR, et al. Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: a randomised controlled trial. *Lancet Oncology* 2009; **10**(11): 1037-44.
17. Brown PD, Asher AL, Ballman KV, et al. NCCTG N0574 (Alliance): A phase III randomized trial of whole brain radiation therapy (WBRT) in addition to radiosurgery (SRS) in patients with 1 to 3 brain metastases. *Journal of Clinical Oncology* 2015; **33**(15).
18. Boogerd W, Dalesio O, Bais EM, Vandersande JJ. RESPONSE OF BRAIN METASTASES FROM BREAST-CANCER TO SYSTEMIC CHEMOTHERAPY. *Cancer* 1992; **69**(4): 972-80.
19. Lin NU, Bellon JR, Winer EP. CNS metastases in breast cancer. *Journal of Clinical Oncology* 2004; **22**(17): 3608-17.
20. Peereboom DM. Chemotherapy in brain metastases. *Neurosurgery* 2005; **57**(5): 54-65.
21. Freilich RJ, Seidman AD, Deangelis LM. CENTRAL-NERVOUS-SYSTEM PROGRESSION OF METASTATIC BREAST-CANCER IN PATIENTS TREATED WITH PACLITAXEL. *Cancer* 1995; **76**(2): 232-6.
22. Rosner D, Nemoto T, Lane WW. CHEMOTHERAPY INDUCES REGRESSION OF BRAIN METASTASES IN BREAST-CARCINOMA. *Cancer* 1986; **58**(4): 832-9.
23. Walbert T, Gilbert MR. The role of chemotherapy in the treatment of patients with brain metastases from solid tumors. *International Journal of Clinical Oncology* 2009; **14**(4): 299-306.
24. Lockman PR, Mittapalli RK, Taskar KS, et al. Heterogeneous Blood-Tumor Barrier Permeability Determines Drug Efficacy in Experimental Brain Metastases of Breast Cancer. *Clinical Cancer Research* 2010; **16**(23): 5664-78.
25. Taskar KS, Rudraraju V, Mittapalli RK, et al. Lapatinib Distribution in HER2 Overexpressing Experimental Brain Metastases of Breast Cancer. *Pharmaceutical Research* 2012; **29**(3): 770-81.
26. Morikawa A, Peereboom DM, Thorsheim HR, et al. Capecitabine and lapatinib uptake in surgically resected brain metastases from metastatic breast cancer patients: a prospective study. *Neuro-Oncology* 2015; **17**(2): 289-95.
27. Lin NU, Carey LA, Liu MC, et al. Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. *Journal of Clinical Oncology* 2008; **26**(12): 1993-9.
28. Lin NU, Dieras V, Paul D, et al. Multicenter Phase II Study of Lapatinib in Patients with Brain Metastases from HER2-Positive Breast Cancer. *Clinical Cancer Research* 2009; **15**(4): 1452-9.
29. Lin NU, Eierman W, Greil R, et al. Randomized phase II study of lapatinib plus capecitabine or lapatinib plus topotecan for patients with HER2-positive breast cancer brain metastases. *Journal of Neuro-Oncology* 2011; **105**(3): 613-20.
30. Bachelot T, Romieu G, Campone M, et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. *Lancet Oncology* 2013; **14**(1): 64-71.
31. Gelmon KA, Boyle FM, Kaufman B, et al. Lapatinib or Trastuzumab Plus Taxane Therapy for Human Epidermal Growth Factor Receptor 2-Positive Advanced Breast Cancer: Final Results of NCIC CTG MA.31. *Journal of Clinical Oncology* 2015; **33**(14): 1574-+.
32. Cameron D, Casey M, Press M, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has

progressed on trastuzumab: updated efficacy and biomarker analyses. *Breast Cancer Research and Treatment* 2008; **112**(3): 533-43.
33. Swain SM, Kim S-B, Cortes J, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncology* 2013; **14**(6): 461-71.
34. Swain SM, Baselga J, Miles D, et al. Incidence of central nervous system metastases in patients with HER2-positive metastatic breast cancer treated with pertuzumab, trastuzumab, and docetaxel: results from the randomized phase III study CLEOPATRA. *Annals of Oncology* 2014; **25**(6): 1116-21.
35. Torres S, Maralani P, Verma S. Activity of T-DM1 in HER-2 positive central nervous system breast cancer metastases. *BMJ case reports* 2014; **2014**.
36. Bartsch R, Berghoff AS, Vogl U, et al. Activity of T-DM1 in Her2-positive breast cancer brain metastases. *Clinical & Experimental Metastasis* 2015; **32**(7): 729-37.
37. Krop IE, Kim S-B, Gonzalez-Martin A, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *Lancet Oncology* 2014; **15**(7): 689-99.
38. Verma S, Miles D, Gianni L, et al. Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer. *New England Journal of Medicine* 2012; **367**(19): 1783-91.
39. Krop IE, Lin NU, Blackwell K, et al. Trastuzumab emtansine (T-DM1) versus lapatinib plus capecitabine in patients with HER2-positive metastatic breast cancer and central nervous system metastases: a retrospective, exploratory analysis in EMILIA. *Annals of Oncology* 2015; **26**(1): 113-9.
40. Carlson JA, Nooruddin Z, Rusthoven C, et al. Trastuzumab emtansine and stereotactic radiosurgery: an unexpected increase in clinically significant brain edema. *Neuro-Oncology* 2014; **16**(7): 1006-9.
41. Lin NU, Amiri-Kordestani L, Palmieri D, Liewehr DJ, Steeg PS. CNS Metastases in Breast Cancer: Old Challenge, New Frontiers. *Clinical Cancer Research* 2013; **19**(23): 6404-18.
42. Canney P, Murray E, Dixon-Hughes J, Lewsley LA, Paul J. A Prospective Randomised Phase III Clinical Trial Testing the Role of Prophylactic Cranial Radiotherapy in Patients Treated with Trastuzumab for Metastatic Breast Cancer - Anglo Celtic VII. *Clinical Oncology* 2015; **27**(8): 460-4.
43. Pivot X, Manikhas A, Zurawski B, et al. CEREBEL (EGF111438): A Phase III, Randomized, Open-Label Study of Lapatinib Plus Capecitabine Versus Trastuzumab Plus Capecitabine in Patients With Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer. *Journal of Clinical Oncology* 2015; **33**(14): 1564-+.
44. Vern-Gross TZ, Lawrence JA, Case LD, et al. Breast cancer subtype affects patterns of failure of brain metastases after treatment with stereotactic radiosurgery. *Journal of Neuro-Oncology* 2012; **110**(3): 381-8.
45. Trudeau ME, Crump M, Charpentier D, et al. Temozolomide in metastatic breast cancer (MBC): A phase II trial of the National Cancer Institute of Canada-Clinical Trials Group (NCIC-CTG). *Annals of Oncology* 2006; **17**(6): 952-6.
46. Addeo R, Caraglia M. Combining temozolomide with other antitumor drugs and target-based agents in the treatment of brain metastases: an unending quest or chasing a chimera? *Expert Opin Investig Drugs* 2011; **20**(7): 881-95.

47. Addeo R, Caraglia M, Faiola V, et al. Concomitant treatment of brain metastasis with whole brain radiotherapy [WBRT] and temozolomide [TMZ] is active and improves quality of life. *BMC Cancer* 2007; **7**: 18.
48. Addeo R, De Rosa C, Faiola V, et al. Phase 2 trial of temozolomide using protracted low-dose and whole-brain radiotherapy for nonsmall cell lung cancer and breast cancer patients with brain metastases. *Cancer* 2008; **113**(9): 2524-31.
49. Addeo R, Sperlongano P, Montella L, et al. Protracted low dose of oral vinorelbine and temozolomide with whole-brain radiotherapy in the treatment for breast cancer patients with brain metastases. *Cancer Chemother Pharmacol* 2012; **70**(4): 603-9.
50. de Azambuja E, Zardavas D, Lemort M, et al. Phase I trial combining temozolomide plus lapatinib for the treatment of brain metastases in patients with HER2-positive metastatic breast cancer: the LAPTEM trial. *Ann Oncol* 2013.
51. Iwamoto FM, Omuro AM, Raizer JJ, et al. A phase II trial of vinorelbine and intensive temozolomide for patients with recurrent or progressive brain metastases. *J Neurooncol* 2008; **87**(1): 85-90.
52. Omuro AM, Raizer JJ, Demopoulos A, Malkin MG, Abrey LE. Vinorelbine combined with a protracted course of temozolomide for recurrent brain metastases: a phase I trial. *J Neurooncol* 2006; **78**(3): 277-80.
53. Rivera E, Meyers C, Groves M, et al. Phase I study of capecitabine in combination with temozolomide in the treatment of patients with brain metastases from breast carcinoma. *Cancer* 2006; **107**(6): 1348-54.
54. Perry JR, Belanger K, Mason WP, et al. Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study. *J Clin Oncol* 2010; **28**(12): 2051-7.
55. Hirst TC, Vesterinen HM, Sena ES, Egan KJ, Macleod MR, Whittle IR. Systematic review and meta-analysis of temozolomide in animal models of glioma: was clinical efficacy predicted? *Br J Cancer* 2013; **108**(1): 64-71.
56. Siena S, Crino L, Danova M, et al. Dose-dense temozolomide regimen for the treatment of brain metastases from melanoma, breast cancer, or lung cancer not amenable to surgery or radiosurgery: a multicenter phase II study. *Annals of Oncology* 2010; **21**(3): 655-61.
57. Palmieri D, Duchnowska R, Woditschka S, et al. Profound Prevention of Experimental Brain Metastases of Breast Cancer by Temozolomide in an MGMT-Dependent Manner. *Clinical Cancer Research* 2014; **20**(10): 2727-39.
58. Bignami M, O'Driscoll M, Aquilina G, Karran P. Unmasking a killer: DNA O-6-methylguanine and the cytotoxicity of methylating agents. *Mutation Research-Reviews in Mutation Research* 2000; **462**(2-3): 71-82.
59. Roos WP, Batista LFZ, Naumann SC, et al. Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O-6-methylguanine. *Oncogene* 2007; **26**(2): 186-97.
60. Hegi ME, Diserens A, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *New England Journal of Medicine* 2005; **352**(10): 997-1003.
61. Pegg AE, Dolan ME, Moschel RC. STRUCTURE, FUNCTION, AND INHIBITION OF O-6-ALKYLGUANINE-DNA ALKYLTRANSFERASE. *Progress in Nucleic Acid Research and Molecular Biology, Vol 51* 1995; **51**: 167-223.
62. Tano K, Shiota S, Collier J, Foote RS, Mitra S. ISOLATION AND STRUCTURAL CHARACTERIZATION OF A CDNA CLONE ENCODING THE HUMAN DNA-REPAIR

PROTEIN FOR O-6-ALKYLGUANINE. *Proceedings of the National Academy of Sciences of the United States of America* 1990; **87**(2): 686-90.
63. Brock CS, Newlands ES, Wedge SR, et al. Phase I trial of temozolomide using an extended continuous oral schedule. *Cancer Research* 1998; **58**(19): 4363-7.
64. de Bono J, Denis L, Patnaik A, et al. Extended temozolomide (TMZ) dosing schedules permit the administration of higher TMZ dose intensities and inhibit the DNA repair enzyme O6-alkylguanine DNA alkyltransferase (AGAT). *European Journal of Cancer* 2001; **37**(Supplement 6): S31-S2.
65. Tolcher AW, Gerson SL, Denis L, et al. Marked inactivation of O-6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *British Journal of Cancer* 2003; **88**(7): 1004-11.
66. Lee SM, Thatcher N, Crowther D, Margison GP. INACTIVATION OF O-6-ALKYLGUANINE-DNA ALKYLTRANSFERASE IN HUMAN PERIPHERAL-BLOOD MONONUCLEAR-CELLS BY TEMOZOLOMIDE. *British Journal of Cancer* 1994; **69**(3): 452-6.
67. Kong D-S, Lee J-I, Kim JH, et al. Phase II trial of low-dose continuous (metronomic) treatment of temozolomide for recurrent glioblastoma. *Neuro-Oncology* 2010; **12**(3): 289-96.
68. Omuro A, Chan TA, Abrey LE, et al. Phase II trial of continuous low-dose temozolomide for patients with recurrent malignant glioma. *Neuro-Oncology* 2013; **15**(2): 242-50.
69. Brastianos PK, Carter SL, Santagata S, et al. Genomic Characterization of Brain Metastases Reveals Branched Evolution and Potential Therapeutic Targets. *Cancer Discovery* 2015; **5**(11): 1164-77.
70. Wang YX, Springer S, Zhang M, et al. Detection of tumor-derived DNA in cerebrospinal fluid of patients with primary tumors of the brain and spinal cord. *Proceedings of the National Academy of Sciences of the United States of America* 2015; **112**(31): 9704-9.
71. De Mattos-Arruda L, Mayor R, Ng CKY, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nature Communications* 2015; **6**: 6.
72. Brastianos PK, Horowitz PM, Santagata S, et al. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. *Nature genetics* 2013; **45**(3): 285-9.
73. Brastianos PK, Taylor-Weiner A, Manley PE, et al. Exome sequencing identifies BRAF mutations in papillary craniopharyngiomas. *Nature genetics* 2014.
74. Shankar GM, Taylor-Weiner A, Lelic N, et al. Sporadic hemangioblastomas are characterized by cryptic VHL inactivation. *Acta Neuropathol Commun* 2014; **2**: 167.
75. Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary human prostate cancer. *Nature* 2011; **470**(7333): 214-20.
76. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature biotechnology* 2013; **31**(3): 213-9.
77. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013; **499**(7457): 214-8.
78. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010; **20**(9): 1297-303.

79. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics* 2009; **25**(21): 2865-71.
80. Carter SL, Cibulskis K, Helman E, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol* 2012.
81. Karayan-Tapon L, Quillien V, Guilhot J, et al. Prognostic value of O6-methylguanosine-DNA methyltransferase status in glioblastoma patients, assessed by five different methods. *J Neurooncol* 2010; **97**: 311-22.
82. Chinot OL, Barrie M, Fuentes S, et al. Correlation between O-6-methylguanine-DNA methyltransferase and survival in inoperable newly diagnosed glioblastoma patients treated with neoadjuvant temozolomide. *Journal of Clinical Oncology* 2007; **25**(12): 1470-5.
83. Brandes AA, Franceschi E, Tosoni A, et al. Temozolomide Concomitant and Adjuvant to Radiotherapy in Elderly Patients With Glioblastoma Correlation With MGMT Promoter Methylation Status. *Cancer* 2009; **115**(15): 3512-8.
84. Brandes AA, Tosoni A, Franceschi E, et al. Recurrence Pattern After Temozolomide Concomitant With and Adjuvant to Radiotherapy in Newly Diagnosed Patients With Glioblastoma: Correlation With MGMT Promoter Methylation Status. *Journal of Clinical Oncology* 2009; **27**(8): 1275-9.
85. Tosoni A, Franceschi E, Ermani M, et al. Temozolomide three weeks on and one week off as first line therapy for patients with recurrent or progressive low grade gliomas. *Journal of Neuro-Oncology* 2008; **89**(2): 179-85.
86. Minniti G, Scaringi C, Arcella A, et al. IDH1 mutation and MGMT methylation status predict survival in patients with anaplastic astrocytoma treated with temozolomide-based chemoradiotherapy. *Journal of Neuro-Oncology* 2014; **118**(2): 377-83.
87. Pietanza MC, Kadota K, Huberman K, et al. Phase II Trial of Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer, with Assessment of Methylguanine- DNA Methyltransferase as a Potential Biomarker. *Clinical Cancer Research* 2012; **18**(4): 1138-45.
88. Hochhauser D, Glynne-Jones R, Potter V, et al. A Phase II Study of Temozolomide in Patients with Advanced Aerodigestive Tract and Colorectal Cancers and Methylation of the O-6-Methylguanine-DNA Methyltransferase Promoter. *Molecular Cancer Therapeutics* 2013; **12**(5): 809-18.
89. Hassel JC, Sucker A, Edler L, et al. MGMT gene promoter methylation correlates with tolerance of temozolomide treatment in melanoma but not with clinical outcome. *British Journal of Cancer* 2010; **103**(6): 820-6.
90. Spitzwieser M, Holzweber E, Pfeiler G, Hacker S, Cichna-Markl M. Applicability of HIN-1, MGMT and RASSF1A promoter methylation as biomarkers for detecting field cancerization in breast cancer. *Breast Cancer Research* 2015; **17**: 13.
91. Kato K, Hara A, Kuno T, et al. Aberrant promoter hypermethylation of p16 and MGMT genes in oral squamous cell carcinomas and the surrounding normal mucosa. *Journal of Cancer Research and Clinical Oncology* 2006; **132**(11): 735-43.
92. Fiano V, Trevisan M, Trevisan E, et al. MGMT promoter methylation in plasma of glioma patients receiving temozolomide. *Journal of Neuro-Oncology* 2014; **117**(2): 347-57.
93. Majchrzak-Celinska A, Paluszczak J, Kleszcz R, et al. Detection of MGMT, RASSF1A, p15INK4B, and p14ARF promoter methylation in circulating tumor-derived DNA of central nervous system cancer patients. *Journal of Applied Genetics* 2013; **54**(3): 335-44.

94. Balana C, Carrato C, Ramirez JL, et al. Tumour and serum MGMT promoter methylation and protein expression in glioblastoma patients. *Clinical & Translational Oncology* 2011; **13**(9): 677-85.
95. Hoffmann AC, Kaifi JT, Vallbohmer D, et al. Lack of Prognostic Significance of Serum DNA Methylation of DAPK, MGMT, and GSTPI in Patients With Non-Small Cell Lung Cancer. *Journal of Surgical Oncology* 2009; **100**(5): 414-7.
96. Yu M, Song S, Nguyen S, Gu HH, Wong E. Cerebrospinal fluid (CSF) O-6-methylguanine-methyltransferase (MGMT) from patients with glioblastoma multiforme. *Neuro-Oncology* 2007; **9**(4): 549-.
97. Palmieri D, Duchnowska R, Woditschka S, et al. Profound Prevention of Experimental Brain Metastases of Breast Cancer by Temozolomide in an MGMT-Dependent Manner. *Clinical Cancer Research* 2014; **20**(10): 2727-39.
98. Fitzgerald D, Palmieri D, Hua E, et al. Reactive glia are recruited by highly proliferative brain metastases of breast cancer and promote tumor cell colonization. *Clin Exp Metast* 2008; **25**: 799-810.
99. Steeg PS, Camphausen KA, Smith QR. Brain metastases as preventive and therapeutic targets. *Nature Reviews Cancer* 2011; **11**(5): 352-63.
100. Freedman RA, Gelman RS, Wefel JS, et al. Translational Breast Cancer Research Consortium (TBCRC) 022: A Phase II Trial of Neratinib for Patients With Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer and Brain Metastases. *Journal of Clinical Oncology* 2016; **34**(9): 945-+.
101. Habets EJJ, Dirven L, Wiggenraad RG, et al. Neurocognitive functioning and health-related quality of life in patients treated with stereotactic radiotherapy for brain metastases: a prospective study. *Neuro-Oncology* 2016; **18**(3): 435-44.
102. Caine C, Deshmukh S, Gondi V, et al. CogState computerized memory tests in patients with brain metastases: secondary endpoint results of NRG Oncology RTOG 0933. *Journal of Neuro-Oncology* 2016; **126**(2): 327-36.
103. Triebel KL, Gerstenecker A, Meneses K, et al. Capacity of patients with brain metastases to make treatment decisions. *Psycho-Oncology* 2015; **24**(11): 1448-55.
104. Fitzgerald DP, Subramanian P, Deshpande M, et al. Opposing Effects of Pigment Epithelium-Derived Factor on Breast Cancer Cell versus Neuronal Survival: Implication for Brain Metastasis and Metastasis-Induced Brain Damage. *Cancer Research* 2012; **72**(1): 144-53.
105. Papa L, Brophy GM, Welch RD, et al. Time Course and Diagnostic Accuracy of Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 in a Large Cohort of Trauma Patients With and Without Mild Traumatic Brain Injury. *Jama Neurology* 2016; **73**(5): 551-60.
106. Brophy GM, Mondello S, Papa L, et al. Biokinetic Analysis of Ubiquitin C-Terminal Hydrolase-L1 (UCH-L1) in Severe Traumatic Brain Injury Patient Biofluids. *Journal of Neurotrauma* 2011; **28**(6): 861-70.
107. Papa L, Silvestri S, Brophy GM, et al. GFAP Out-Performs S100 beta in Detecting Traumatic Intracranial Lesions on Computed Tomography in Trauma Patients with Mild Traumatic Brain Injury and Those with Extracranial Lesions. *Journal of Neurotrauma* 2014; **31**(22): 1815-U11.
108. Papa L, Brophy GM, Hannay HJ, et al. EARLY CSF LEVELS OF UCH-L1 ARE ASSOCIATED WITH MEASURES OF INJURY SEVERITY IN PATIENTS WITH SEVERE TBI. *Journal of Neurotrauma* 2012; **29**(10): A143-A.

109. Ren CH, Kobeissy F, Alawieh A, et al. Assessment of Serum UCH-L1 and GFAP in Acute Stroke Patients. *Scientific Reports* 2016; **6**: 9.
110. Zovoilis A, Agbemenyah HY, Agis-Balboa RC, et al. microRNA-34c is a novel target to treat dementias. *Embo Journal* 2011; **30**(20): 4299-308.
111. Bhatnagar S, Chertkow H, Schipper HM, et al. Increased nnicroRNA-34c abundance in Alzheimer's disease circulating blood plasma. *Frontiers in Molecular Neuroscience* 2014; **7**: 11.
112. Pacifici M, Delbue S, Kadri F, Peruzzi F. Cerebrospinal Fluid MicroRNA Profiling Using Quantitative Real Time PCR. *Jove-Journal of Visualized Experiments* 2014; (83): 7.
113. Hoshino A, Costa-Silva B, Shen TL, et al. Tumour exosome integrins determine organotropic metastasis. *Nature* 2015; **527**(7578): 329-+.
114. Armstrong TS, Gning I, Mendoza TR, et al. Clinical Utility of the MDASI-BT in Patients with Brain Metastases. *Journal of Pain and Symptom Management* 2009; **37**(3): 331-40.
115. Saffer BY, Lanting SC, Koehle MS, Klonsky ED, Iverson GL. Assessing cognitive impairment using PROMIS (R) applied cognition-abilities scales in a medical outpatient sample. *Psychiatry Research* 2015; **226**(1): 169-72.
116. Lin NU, Lee EQ, Aoyama H, et al. Response assessment criteria for brain metastases: proposal from the RANO group. *Lancet Oncology* 2015; **16**(6): E270-E8.
117. Kased N, Binder DK, McDermott MW, et al. GAMMA KNIFE RADIOSURGERY FOR BRAIN METASTASES FROM PRIMARY BREAST CANCER. *International Journal of Radiation Oncology Biology Physics* 2009; **75**(4): 1132-40.

15 APPENDICES

15.1 APPENDIX A – PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

15.2 APPENDIX B – LIST OF DRUGS INTERACTING WITH CYP3A4 ISOENZYME

CYP3A4 INHIBITORS		
Strong Inhibitors (prohibited)	**Moderate Inhibitors** (use with caution, avoid if possible)	**Weak Inhibitors** (use with caution, avoid if possible)
Amprenavir1	Amiodarone1	Chloramphenicol2
Atazanavir[1]	Aprepitant	Ciprofloxacin[2]
Clarithromycin	Cimetidine[1]	Diethyldithiocarbamate[2]
Conivaptan[1]	Clotrimazole[1]	Fluvoxamine[2]
Delavirdine[1]	Cyclosporine[1]	Gestodene[2]
Fosamprenavir[1]	Desipramine[1]	Mibefradil[2]
Fospropofol[1]	Doxycycline[1]	Mifepristone
Imatinib[1]	Efavirenz[1]	Norfluoxetine[2]
Indinavir	Erythromycin	Star fruit[2]
Isoniazid[1]	Fluconazole	Troleandomycin[2]
Itraconazole	Fosaprepitant[1]	
Ketoconazole	Grapefruit juice	
Miconazole[1]	Haloperidol[1]	
Nefazodone	Lidocaine[1]	
Nelfinavir	Metronidazole[1]	
Nicardipine[1]	Norfloxacin[1]	
Posaconazole[1]	Sertraline[1]	
Propofol[1]	Tetracycline[1]	
Quinidine[1]	Verapamil	
Ritonavir	Voriconazole[1]	
Saquinavir[2]		
Telithromycin		

1 Cited in Cytochrome P450 Enzymes: Substrates, Inhibitors, and Inducers. In: Lacy CF, Armstrong LL, Goldman MP, Lance LL, eds. Drug Information Handbook 20th ed. Hudson, OH; LexiComp Inc. 2011-2012: 1810-1818
2 Cited in Flockhart DA. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine (2007). http://medicine.iupui.edu/clinpharm/ddis/table.asp. Accessed Nov 2011. Note: Drugs without a superscript are cited in both the Lacy and Flockhart references.

15.3 APPENDIX C – ORAL MEDICATION DIARY

TEMOZOLOMIDE

Today's Date _____ Cycle #_____
Patient Name_____ Patient Study ID _____
(initials acceptable for patien's name)

INSTRUCTIONS TO THE PATIENT:
1. Complete one form for each cycle (21 days).
2. You will take ___ (number capsule(s)), total _____ mg (dosage) each day.
3. Please, do not eat anything for at least 2 hours before and 1 hour after temozolomide administration.
4. Record the date, the number of pills you took, and when you took them.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. Please bring your pill bottle and this form to your physician when you go for your next appointment.

Date	**Day**	**# pills and when taken: temozolomide**		**Comments**	**Date**	**Day**	**# pills and when taken: temozolomide**		**Comments**
		Dose	**Time**				**Dose**	**Time**	
	1					12			
	2					13			
	3					14			
	4					15			
	5					16			
	6					17			
	7					18			
	8					19			
	9					20			
	10					21			
	11								

Study Team will complete this section:
1. Date patient started protocol treatment_____
2. Date patient was removed from study _____
3. Patient's planned daily dose_____
4. Total number of pills taken this month _____

Physician/Nurse's Signature: _____

15.4 APPENDIX D– RANO CRITERIA FOR CNS METASTASIS

Panel 1: **Response assessment of target and non-target lesions**

Target lesions

Complete response

Disappearance of all CNS target lesions sustained for at least 4 weeks; with no new lesions, no use of corticosteroids, and patient is stable or improved clinically.

Partial response

At least a 30% decrease in the sum longest diameter of CNS target lesions, taking as reference the baseline sum longest diameter sustained for at least 4 weeks; no new lesions; stable to decreased corticosteroid dose; stable or improved clinically.

Progressive disease

At least a 20% increase in the sum longest diameter of CNS target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, at least one lesion must increase by an absolute value of 5 mm or more to be considered progression.

Stable disease

Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter while on study.

Non-target lesions

Non-target lesions should be assessed qualitatively at each of the timepoints specified in the protocol.

Complete response

Requires all of the following: disappearance of all enhancing CNS non-target lesions, no new CNS lesions.

Non-complete response or non-progressive disease

Persistence of one or more non-target CNS lesion or lesions.

Progressive disease

Any of the following: unequivocal progression of existing enhancing non-target CNS lesions, new lesion(s) (except while on immunotherapy-based treatment), or unequivocal progression of existing tumour-related non-enhancing (T2/FLAIR) CNS lesions. In the case of immunotherapy-based treatment, new lesions alone may not constitute progressive disease.

Lin NU, Lee EQ, Aoyama H, et al. Response assessment criteria for brain metastases: proposal from the RANO group. *Lancet Oncology* 2015; **16**(6): E270-E8

	Complete response	Partial response	Stable disease	Progressive disease
Target lesions	None	≥30% decrease in sum longest distance relative to baseline	<30% decrease relative to baseline but <20% increase in sum longest distance relative to nadir	≥20% increase in sum longest distance relative to nadir*
Non-target lesions	None	Stable or improved	Stable or improved	Unequivocal progressive disease*
New lesion(s)†	None	None	None	Present*
Corticosteroids	None	Stable or decreased	Stable or decreased	Not applicable‡
Clinical status	Stable or improved	Stable or improved	Stable or improved	Worse*
Requirement for response	All	All	All	Any‡

*Progression occurs when this criterion is met. †A new lesion is one that not present on prior scans and is visible in minimum two projections. If a new lesion is equivocal, for example because of its small size, continued therapy can be considered, and follow-up assessment will clarify if the new lesion is new disease. If repeat scans confirm there is definitely a new lesion, progression should be declared using the date of the initial scan showing the new lesion. For immunotherapy-based approaches, new lesions alone to do not define progression. ‡Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

Table 2: **Summary of the response criteria for CNS metastases proposed by RANO-BM**

Lin NU, Lee EQ, Aoyama H, et al. Response assessment criteria for brain metastases: proposal from the RANO group. *Lancet Oncology* 2015; **16**(6): E270-E8