

North Central Cancer Treatment Group

N0776: Phase II Trial of Avastin® in Combination with Sorafenib in Recurrent Glioblastoma Multiforme

Addendum 1 – January 22, 2009

Summary

- In order to compare the efficacy to historical data for this patient population, a new inclusion criteria has been added to allow up to 1 prior chemotherapy regimen for progressive/recurrent disease.
- A new inclusion criteria has been added to reflect that patients must be on stable corticosteroid dosing or no corticosteroids in order to ensure accuracy of baseline scans.
- Administrative/editorial changes.

A replacement protocol is provided. Please replace the current copy with the one attached. Please keep this addendum with your protocol

Title page Updated to reflect the addition of Addendum 1 and revised NCI version date.

Protocol Resource Page

Page 2: **Wanda DeKrey, R.N., OCN** replaces ~~Beverly L. Kowbel, R.N., B.S.C.N.~~ as the NCCTG Member Nurse.

The title for Janis Wobschall has been updated as follows:
NCCTG *Research Base* **Research** Protocol ~~Development Coordinator~~ **Specialist**

Section 3.0 **Patient Eligibility**

Pages 7-8: In order to remain consistent with this patient population, a new inclusion criteria has been added as follows and all remaining sections renumbered:

3.26 ≤1 chemotherapy regimen for progressive/recurrent disease.

Page 8: Section 3.29g is newly added as follows to ensure accuracy of baseline scans:
Fixed dose of corticosteroids (or no corticosteroids) ≥1 week prior to baseline scan.

Page 8: The last sentence of Section 3.38 has been revised for clarification as follows:
Therapeutic anti-coagulation with low molecular weight heparin is allowed at time of registration **and during the study if needed.**

Section 4.0 **Test Schedule**

Page 11: Footnote #11 has been revised for clarification as follows:
At baseline, Cycle 1 Day 3 (**±1 day**), prior to the 3rd cycle (Day 28) (**±2 days**), prior to the 5th cycle of treatment (**±2 days**). DCE component in subsequent MRIs is optional (see Section 4.2).

Section 6.0

Registration/Randomization Procedures

Page 13:

In Section 6.22, reference to Section 3.29e has been corrected as follows:
(Sections 3.29ef and 14.0)

Section 10.0

Adverse Event (AE) Reporting and Monitoring

Page 24:

Under the Section “Additional Instructions or Exceptions to AdeERS Expedited Reporting Requirements” in Section 10.21, the third bullet has been revised as follows at the request of Bayer/Onyx:

Bayer/Onyx: DrugSafety.GPV.US@bayer.com (within 24 hours)

Section 11.0

Treatment Evaluation

Page 27:

The tables in Section 11.14 have been revised as follows to delete “NED” as this is not being used in this study:

NEURO STATUS	MRI and/or CT Status					
	NED	CR	PR	REGR	SD	PD
Better	NED	CR	PR	REGR	SD	UNKN*
Same						PD
Worse						UNKN*

* Set the Objective Status equal to unknown. Treat one more cycle and evaluate according to the table below:

NEURO STATUS	MRI and/or CT Status					
	NED	CR	PR	REGR	SD	PD
Better	NED	CR	PR	REGR	SD	PD
Same						
Worse						

Section 14.0

Body Fluid Biospecimens

Page 29:

Section 14.22 has been corrected as follows:

All samples must be collected Monday-~~Friday~~ **Thursday ONLY**.

Page 31:

The last sentence of Section 14.254 has been revised for clarification as follows:
Ship specimens via Priority Overnight service, Monday – ~~Friday~~ **Thursday ONLY**, to Mayo Clinical Trial Services (MCTS. Do not send samples on **Fridays**, weekends or holidays.

Pages 31-32:

Section 14.11 has been revised to remove unnecessary details regarding methodology. References containing the information are cited.

Analysis of circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPCs) will be performed at baseline, Cycle 1 Day 3, prior to treatment Cycle 2, prior to treatment Cycle 3, and prior to treatment every 4 weeks thereafter x 5 (i.e., prior to treatment Cycles 5, 7, 9, 11, and 13). Analysis has to be performed, at the latest, ≤48 hours from sample collection for the

results to be valid. **Samples must be collected Monday-Thursday and shipped overnight.**

Analyses of CECs and CEPs will be performed in the laboratory of Dr. Shaji Kumar, Stable 6-13, Mayo Clinic Rochester. Whole blood samples will be collected in a 2 x 10 mL vacutainer EDTA tubes at the time points indicated above. Enumeration using four-color flow cytometry (Khan, 2005 et al.; Mancuso et al., 2003). Phenotypic markers used to identify the two populations will be as follows: CEPs CD45⁻ to exclude hematopoietic cells, CD13⁺ VEGFR-2⁺/AC133⁺. Similarly, CECs as CD45⁻/CD13⁺/VEGFR-2⁺ will be assayed. Whole blood samples will be diluted 1:2 in Hanks Balanced Solution with 0.5 M EDTA, 0.5% bovine albumin. Diluted samples will be added to Ficoll-Histopaque plus 20 ml in a 50 ml conical tube. Sample will be centrifuged at 1400Xg for 30 min. The mononuclear layer will be removed by pipetting and placed in a separate conical tube. Cells will be washed once in PBS and centrifuged; the supernatant will be removed and discarded. The pellet will be re-suspended in 100 ul FACS buffer solution containing PBS and 3% normal goat serum.

To each tube, monoclonal antibodies anti-VEGFR2/KDR (R&D systems), anti-AC133-phycoerythrin (Miltenyi Biotech), anti-CD45-peridinin-chlorophyll protein-Cy5.5 (BD Biosciences), anti-CD13-allophycocyanin (BD Biosciences). Optimal antibody concentration will be determined prior to running patient samples, as determined by analysis of control samples. For each fluorescein dye, an appropriate isotype IgG conjugated control will be used as a negative control. Following a one-hour incubation, samples will be centrifuged, with removal of supernatant and undergo three subsequent washes with PBS. After each wash, samples will be centrifuged and supernatant aspirated.

For primary antibodies with a conjugated fluorochrome, samples will be suspended directly in 0.5 ml of 2% paraformaldehyde fixation and stored at 4°C, protected from light until analysis. For unconjugated antibodies, an incubation of 30 minutes with the appropriate secondary, conjugated to a fluorochrome will be performed. After incubation, samples will be washed thrice in PBS as noted above and fixed in 0.5 ml 2% paraformaldehyde and stored at 4 degrees Celsius until analysis. Flow cytometry will be performed with twelve hours of sample fixation.

Flow cytometry will be performed with evaluation on FACSCalibur cell analyzer and Cellquest Pro acquisition program using analysis gates designed to exclude dead cells, platelets and debris. Acquisition of 100,000 events per sample will be obtained in order to analyze the percentage of CEPs/CECs as defined according to surface receptor profile given above. Percentage of stained cells will be determined based on positive staining defined as greater than 98% of nonspecific background staining according to each isotype-fluorescein conjugated control.

CECs and their progenitor subpopulation (CEPs) will be evaluated as previously described (Mancuso, 2001; Khan, 2005). In brief, a minimum of eight ml of whole blood will be lysed to remove RBC (red blood cells). A portion of the cells will be stained in BD Pharmingen Trucount© tubes for

the absolute count calculation of endothelial cells by their characteristic low FWD/SSC and CD146+CD3-CD31 + phenotype. Cells will also be stained with CD133 or CD105 to evaluate the percentages of progenitor (CD133+) or activated (CD105+) endothelial cell subsets. Isotype control reagents will be included to account for non-specific staining and 7 AAD will be used to exclude dead cells from further analysis.

Pages 32-62: Due to the deletion of text in Section 14.411, repagination has occurred.

Section 17.0

Pathology Considerations/Tissue Biospecimens

Page 56:

The first column of the second row in Section 17.1 has been corrected as follows:
Formalin-fixed paraffin-embedded (FFPE) tissue blocks with corresponding H&E ~~or~~ **OR** 10 unstained slides with 2 corresponding H&Es)

Page 57:

Section 17.22 has been updated as follows to reflect current template language:

17.22 The following materials below are mandatory (unless indicated otherwise) and required for shipment:

- Diagnostic slides from all surgical biopsies
- Pathology Reporting Form
- **Pathology Submission Form**
- Surgical Pathology Report
- Operative Report (*optional*)

The address in Section 17.262 has been updated as follows:

NCCTG Operations Office
Attn: NCCTG PC Office (**Study N0776**)
RO_FF_03_24-CC/NW Clinic
200 First Street SW
Rochester, MN 55905

Page 59:

The address in Section 17.37 has been updated as follows:

NCCTG Operations Office
Attn: NCCTG PC Office (**Study N0776**)
RO_FF_03_24-CC/NW Clinic
200 First Street SW
Rochester, MN 55905

Section 18.0

Records and Data Collection Procedures

Page 60:

For the row “Blood Pressure and Pulse Form,” the words “and Pulse” have been deleted as pulse is not collected in this study.

Section 20.0

Page 62:

References

Due to the revisions in Section 14.411, the following references are newly added:

Mancuso P, Burlini A, Pruneri G, et al. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood. 2001;97: 3658-3661

Khan, Solomon, and McCoy. Detection of Circulating Endothelial Cells and Endothelial progenitor Cells by Flow Cytometry. Cytometry Part B (Clinical Cytometry)64B:1-8 (2005)

Appendix I

Page 3:

Consent Form

The fourth paragraph under “During the study” section has been corrected as follows:

For cycle 1, you will take sorafenib, ~~two~~ **one** tablets by mouth twice a day on days 1 through 5 and 8 through 12. If you do not have any bad side effects during cycle 1, you will take sorafenib, ~~two~~ **one** tablets by mouth twice a day every day. If you do have any bad side effects but they are not bad enough for you to stop treatment, you will continue to take the sorafenib days 1 through 5 and 8 through 12 during each cycle of treatment. A cycle of treatment covers a 14-day period of time. The treatment will continue until your disease gets worse.

Page 4:

The last sentence of the second to the last paragraph under the section “During the study” has been revised for clarification as follows:

Blood samples will be collected before treatment begins, Cycle 1 Day 3, before treatment Cycle 2, before treatment Cycle 3, and then before treatment every 4 weeks times 5 (**before treatment Cycles 5, 7, 9, 11, and 13**).

Page 4:

The following text is being deleted in the last paragraph under the section “During the study” as this information is also reflected in the second paragraph under the section “Please read the following statements and mark your choice:

This study also has optional laboratory tests that will be done to study small samples of tissue. You are or will have had a biopsy to see if your cancer has returned. ~~Your doctor will use some of this body tissue to do some tests. The results of these tests will not be given to you by your doctor and will not be used to plan your care.~~ No additional biopsies will be done to get this tissue.

Page 5: In the section “When I am finished taking sorafenib and Avastin®,” the first table “Cycle 1 (14 days)” has been corrected as follows:

Day	What you do
Day 1	<ul style="list-style-type: none"> • Begin taking sorafenib twice a day. • Avastin® will be given into a vein. • Blood pressure check <p><i>Add in the following for Mayo Rochester patients only: Research MRI</i></p>
Day 3	<ul style="list-style-type: none"> • Come back to the Clinic to get a research blood draw. <p><i>Add in the following for Mayo Rochester patients only: Research MRI</i></p>
Days 1-5 and 8-12	<ul style="list-style-type: none"> • Take the sorafenib on day 1 through day 5 then no sorafenib on day 6 through day 7 and then start again on day 8 through day 12.
Day 14	<ul style="list-style-type: none"> • Return to your doctor’s office at _____ [<i>insert appointment time</i>] for your next exam and to begin the next cycle. • Bring pill diary to this appointment.

Page 5 The first paragraph under the section “How long will I be in the research study?” has been clarified as follows:

You will be asked to take sorafenib and Avastin® until your tumor gets worse. After that, we would like to keep track of your medical condition ~~for about 15 years~~ **indefinitely**. Keeping in touch with you and checking on your condition ~~every year~~ helps us look at the long-term effects of the study.

Appendix III Patient Medication Diary

Has been revised for clarification as follows:

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each ~~month~~ **cycle**.
2. You will take two tablets each day, **one in the morning and one in the evening**. Tablets need to be swallowed whole with about 8 ounces of water. ~~Take one tablet in the morning and one tablet in the evening.~~ Tablets may be taken with or without food. Tablets should not be taken with grapefruit/grapefruit juice. If you miss a dose, do not make it up.
3. Record the date, ~~the number of tablets you took,~~ and **the time** when you took ~~them~~ **the tablet**.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring your tablet bottle and this form to your physician when you go for your next appointment.

Day	Date	#tablets and when taken Time morning tablet was taken	Time evening tablet was taken	Comments
1	1	a.m.	p.m.	
2	2	a.m.	p.m.	
3	3	a.m.	p.m.	
4	4	a.m.	p.m.	
5	5	a.m.	p.m.	
6	6	a.m.	p.m.	
7	7	a.m.	p.m.	
8	8	a.m.	p.m.	
9	9	a.m.	p.m.	
10	10	a.m.	p.m.	
11	11	a.m.	p.m.	
12	12	a.m.	p.m.	
13	13	a.m.	p.m.	
14	14	a.m.	p.m.	

Patient's Signature: _____ Date: _____

Physician's Office will complete this section:

1. Date patient started protocol treatment _____ Date patient was removed from study _____
2. Patient's planned daily dose _____ Total number of pills taken this month _____

Physician/Nurse/Data Manager's Signature _____

Appendix VII

Page 2:

Research Base Instructions for Biospecimen Processing in BAP Laboratory

Footnote #6 has been updated as follows:

At the end of the study, forward one frozen plasma aliquot (aliquot volume to be determined at the end of the study) to the laboratory of Dr. Shaji Kumar, Stable 6-13, Mayo Clinic Rochester (ATTN: ~~Jessica Haug Terry~~ **Kimlinger**) for the assaying of circulating VEGF, PlGF, soluble VEGFR1, bFGF, soluble VEGFR2, and SDF1- α by ELISA.