

RADIATION THERAPY ONCOLOGY GROUP

RTOG 0611

**URINARY VEGF AND MMP LEVELS IN PATIENTS RECEIVING RADIATION THERAPY
FOR GLIOBLASTOMA MULTIFORME: PROSPECTIVE DETERMINATION OF A
PREDICTIVE VALUE FOR RECURRENCE**

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INDEX

	Eligibility Checklist
1.0	Introduction
2.0	Objectives
3.0	Patient Selection
4.0	Pre-treatment Evaluations
5.0	Registration Procedures
6.0	Treatment Evaluation
7.0	Drug Therapy
8.0	Surgery
9.0	Other Therapy
10.0	Tissue/Specimen Submission
11.0	Patient Assessments
12.0	Data Collection
13.0	Statistical Considerations
	References
	Appendix I - Sample Consent Form

Required Sample Size: 200 (5/25/07)

RTOG Institution # _____

RTOG 0611

ELIGIBILITY CHECKLIST (2/2/06)

Case # _____

(page 1 of 2)

- _____ (Y) 1. Is the patient enrolled on an RTOG GBM treatment study that prescribes 6000 cGy of radiation therapy?
- _____ 2. On what RTOG treatment study is the patient enrolled?
- _____ 3. What is the patient's case number on the RTOG treatment study?

The following questions will be asked at Study Registration:

- _____ 1. Name of institutional person registering this case?
- _____ (Y) 2. Has the Eligibility Checklist (above) been completed?
- _____ (Y) 3. Is the patient eligible for this study?
- _____ 4. Date the study-specific Consent Form was signed? (must be prior to study entry)
- _____ 5. Patient's Initials (First Middle Last)
- _____ 6. Verifying Physician
- _____ 7. Patient's ID Number
- _____ 8. Date of Birth
- _____ 9. Race
- _____ 10. Ethnic Category (Hispanic or Latino; Not Hispanic or Latino; Unknown)
- _____ 11. Gender
- _____ 12. Patient's Country of Residence
- _____ 13. Zip Code (U.S. Residents)

RTOG Institution # _____

RTOG 0611

ELIGIBILITY CHECKLIST (2/2/06)

Case # _____

(page 2 of 2)

14. Patient's Insurance Status

15. Will any component of the patient's care be given at a military or VA facility?

16. Treatment Start Date

17. RTOG treatment study on which the patient is currently enrolled

18. Patient case number from RTOG treatment study on which the patient is currently enrolled

The Eligibility Checklist must be completed in its entirety prior to web registration. The completed, signed, and dated checklist used at study entry must be retained in the patient's study file and will be evaluated during an institutional NCI/RTOG audit.

Completed by _____

Date _____

1.0 INTRODUCTION

1.1 Angiogenesis

Tumor growth depends on angiogenesis, the development and recruitment of new blood vessels. The importance of angiogenesis in oncology was proposed by Folkman, who recognized the significance of targeting cells that support tumor growth rather than the cancer cells themselves.¹ Despite the array of tumor markers currently in use, none serve as general cancer predictors of outcome. In theory, angiogenic factors could identify patients at risk for recurrent disease, regardless of tumor type since the process of angiogenesis is ubiquitous to cancer. Indeed, multiple investigators have explored the use of angiogenic factors as possible general tumor markers.²⁻¹⁵ While angiogenic proteins such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinase (MMPs), have been shown to have prognostic power in specific tumor types, few studies have individually explored the utility of angiogenic proteins as general tumor markers across different tumor types—both solid tumors and hematologic malignancies. A tumor marker that could consistently identify patients at risk for non-responsive or recurrent disease would allow selection of these patients for more aggressive or alternate treatment.

Furthermore, to our knowledge each of the aforementioned studies has focused on the magnitude of either the VEGF or MMP initial level. No study has explored the dynamic *trend* of these protein levels though a course of therapy, its possible predictive significance, or evaluated which methodology was the most powerful for defining the trend.

1.2 VEGF

VEGF, originally known as vascular permeability factor, is one of the most potent and well-characterized pro-angiogenic proteins. It is expressed by a variety of human solid tumors, as well as neoplastic myeloid and leukemic cells.¹⁶ VEGF has specific mitogenic activity on endothelial cells and promotes extravasation of proteins from tumor vessels, which creates a fibrin matrix infrastructure allowing for stromal cell invasion and tumor development.¹⁷

1.3 MMPs

MMPs are a family of zinc dependent enzymes that enable new vessels to invade the surrounding extracellular matrix. MMPs function during the normal physiologic processes of tissue repair and morphogenesis. Pathologically, MMPs have been implicated in diseases associated with excess degradation of extracellular matrix, such as rheumatoid arthritis, osteoarthritis, periodontitis, autoimmune skin disorders, tumor invasion and metastasis.¹⁸ Increased MMP expression has been linked to more aggressive metastatic behavior, and increased expression of MMPs has been documented in numerous tumor types.¹⁹

1.4 Glioblastoma Multiforme

While advances in surgical management and radiation therapy have improved survival for patients with high-grade gliomas, life expectancy remains poor. Overall, patients diagnosed with glioblastoma multiforme (GBM) can expect a 9% 2-year survival. However, within a cohort of patients diagnosed with GBM, a significantly different clinical course may occur, with some patients surviving weeks following diagnosis and others surviving years, independent of treatment undertaken.

This heterogeneity in clinical outcomes led to a desire to stratify patients into risk categories that allow appropriate comparisons in randomized trials and comparisons to historical controls in phase II trials. Multiple groups have retrospectively evaluated clinical variables that might predict for outcome following diagnosis with a high-grade glioma.

The RTOG utilized a recursive partitioning technique to evaluate the association of pre-treatment patient, tumor, and treatment variables with survival duration.²⁰ In this study, high-grade histology, age, performance status, extent of surgery, and mental status at presentation were found to be significant predictors of survival.²⁰ Multiple groups have confirmed these clinical variables as prognostic for tumor recurrence or survival.²¹⁻²⁵ Ongoing trials in high-grade gliomas are using the RTOG recursive partitioning prognostic subgroups for stratification.

Recently, the RTOG has revised its recursive partition analysis for 1672 patients with GBM to include only four variables: age, Karnofsky performance status (KPS), extent of resection (gross total or subtotal vs. biopsy) and neurologic function (working vs. not working). The new prognostic groups for GBM are defined below:

RPA class	Definition	Median Survival	1-year Survival	3-year Survival	5-year Survival
III	Age<50, KPS≥90	17.1 mos	70%	20%	14%
IV	Age<50, KPS<90	11.2 mos	46%	7%	4%
	Age≥50, KPS≥70 G/STR, Working				
V and VI	Age≥50, KPS≥70 G/STR, Not work	7.5 mos	28%	1%	0%
	Age≥50, KPS≥70 Biopsy				
	Age≥50, KPS<70				

While clinical prognostic variables have assisted in stratifying patients, many groups are investigating molecular prognostic factors with the hope that these markers might also predict for response to specific therapies. Specific genetic markers in pathology specimens have been found to be prognostic in gliomas and predict responsiveness to chemotherapy.²⁶⁻²⁹ Expression of growth factors and the proliferation index have also been found to be prognostic in tumor tissue from patients with high-grade gliomas.

The majority of studies evaluating molecular prognostic variables with gliomas have focused on the evaluation of tumor tissue obtained at the time of biopsy and/or resection. This approach is well suited to evaluate molecular pre-treatment prognostic markers.

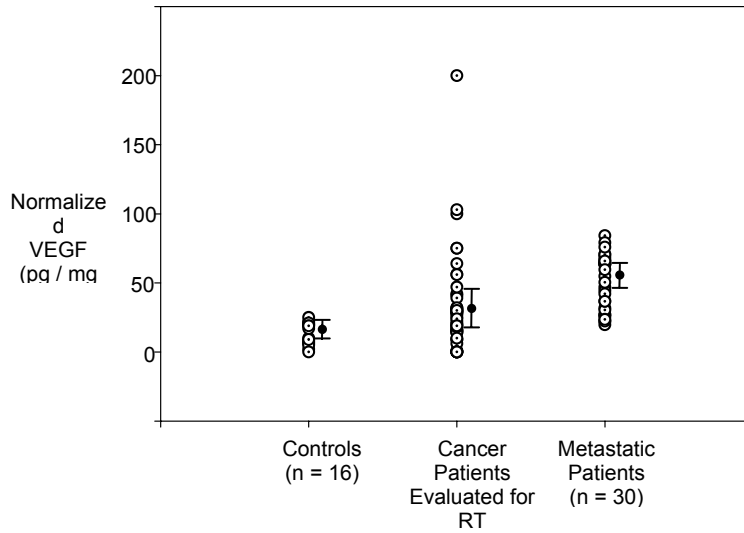
The investigation of predictive post-treatment factors in patients with gliomas has been more limited, due to the paucity of available tumor tissue after therapy. Evaluating prognostic markers in urine and serum, which can be obtained with minimally invasive procedures, may be more feasible and applicable to this setting.

1.5 Previous Data

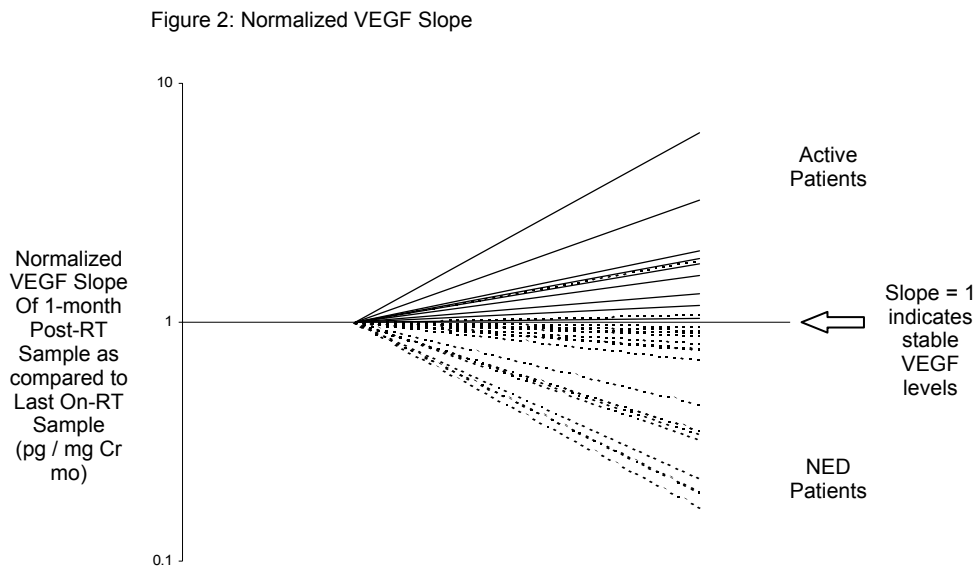
The NCI Radiation Oncology Branch (ROB) previously evaluated serum and urine markers prior to, during, and following definitive therapy in patients undergoing radiation therapy, including several patients with high-grade gliomas.³⁰ These patients were enrolled in the ROB blood and urine collection protocol, (NCI protocol 02-C-0064). Serum and urine were collected from patients with various cancers undergoing radiation therapy. The levels of serum and urine angiogenic factors were evaluated to determine if these levels were prognostic of outcome following radiation therapy.

Urinary VEGF level at presentation were different between patients with local-regional cancer and normal controls, and between patients with metastatic prostate cancer and local-regional disease, ($p = 0.04$ and 0.01 , respectively, Figure 1). Similar results were found with MMP measurement ($p = 0.03$ and $p < 0.0001$, respectively).

Figure 1: Normalized VEGF levels at initial presentation



Of those patients subsequently treated with radiation, VEGF levels at presentation between patients with no evidence of disease after radiation (NED) and those who had persistent or recurrent disease following radiotherapy were also different ($p = 0.039$). The comparison between angiogenic factor levels taken at least 1 month post-radiotherapy and the last on-treatment level was the strongest predictor of patient 1-year progression-free survival ($p = 0.004$). Similarly, overall MMP trending was also significantly associated with one-year progression-free survival, as was individual MMP-2 trending ($p = 0.004$ and 0.001 , respectively). Step-wise logistic regression revealed that the VEGF trend comparing post-radiation levels to last on-treatment levels was an independent predictor of progression-free survival ($p = 0.02$) (Figure 2).



As our previous study was exploratory in nature the 95% confidence levels for our tests were quite large (Table I).

Table 1: Statistical Performance Characteristics for VEGF and MMP

<i>Trend</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>Positive Likelihood Ratio</i>	<i>Negative Likelihood Ratio</i>
Dur RT VEGF	80%	48%	1.54	0.42
Dur RT Overall MMP	80%	42%	1.37	0.48
Post RT VEGF	86%	72%	3.06	0.20
Post RT Overall MMP	86%	79%	4.11	0.18
Post RT HMW-MMP	57%	79%	2.74	0.54
Post RT MMP-121	43%	83%	2.57	0.69
Post RT MMP-9	57%	83%	3.43	0.51
Post RT MMP-2	100%	71%	3.43	--

These values are based on the ability of each measurement to discriminate between the active and NED disease status. Dur RT represents the trend comparing the last on-treatment VEGF or MMP level as compared to the VEGF or MMP level at presentation. Post RT represents the trend comparing the VEGF or MMP level one-month post-RT, as compared to the last on-treatment VEGF or MMP level. Note that Post LMW-MMP was omitted from the table due to too few samples for meaningful calculation.

Therefore, we plan to expand on these results by prospectively investigating this question in patients with GBM.

1.6 Conclusion

This study will aim to determine whether urinary VEGF and MMP levels are predictive of clinical outcome in patients receiving radiotherapy for GBM.

2.0 OBJECTIVES

- 2.1 To determine if an increase in urinary VEGF and MMP level, from the end of treatment to a patient's 1-month follow-up examination following radiotherapy is predictive of 1-year recurrence in patients with GBM.

3.0 PATIENT SELECTION

3.1 Conditions for Patient Eligibility

- 3.1.1 Patient must be enrolled on an RTOG GBM study that prescribes 6000 cGy of radiation therapy.
- 3.1.2 Patient must meet the eligibility requirements for the RTOG treatment study. (If the patient is deemed retrospectively ineligible for the RTOG treatment study, the patient will likewise be ineligible for this study.)
- 3.1.3 Patient must sign a study-specific informed consent for RTOG 0611 prior to study entry.

3.2 Conditions for Patient Ineligibility

- 3.2.1 Patient not able to receive 6000 cGy of radiation therapy.

4.0 PRE-TREATMENT EVALUATIONS

Not applicable to this study.

5.0 REGISTRATION PROCEDURES

Patients can be registered only after eligibility criteria are met.

Institutions must have an RTOG user name and password to register patients on the RTOG web site. To get a user name and password:

- The Investigator must have completed Human Subjects Training and been issued a certificate (Training is available via <http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp>).
- The institution must complete the Password Authorization Form at www.rtog.org/members/webreg.html (bottom right corner of the screen), and fax it to 215-923-1737. RTOG Headquarters requires 3-4 days to process requests and issue user names/passwords to institutions.

An institution can register the patient by logging onto the RTOG web site (www.rtog.org), going to "Data Center Login" and selecting the link for new patient registrations. The system triggers a program to verify that all regulatory requirements (OHRP assurance, IRB approval) have been met by the institution. The registration screens begin by asking for the date on which the eligibility checklist was completed, the identification of the person who completed the checklist, whether the patient was found to be eligible on the basis of the checklist, and the date the study-specific informed consent form was signed.

Once the system has verified that the patient is eligible and that the institution has met regulatory requirements, it assigns a patient-specific case number. The system then moves to a screen that confirms that the patient has been successfully enrolled. This screen can be printed so that the registering site will have a copy of the registration for the patient's record. Two e-mails are generated and sent to the registering site: the Confirmation of Eligibility and the patient-specific calendar. The system creates a case file in the study's database at the DMC (Data Management Center) and generates a data submission calendar listing all data forms, images, and reports and the dates on which they are due.

If the patient is ineligible or the institution has not met regulatory requirements, the system switches to a screen that includes a brief explanation for the failure to register the patient. This screen can be printed.

In the event that the RTOG web registration site is not accessible, participating sites can register a patient by calling RTOG Headquarters, at (215) 574-3191, Monday through Friday, 8:30 a.m. to 5:00 p.m. ET. The registrar will ask for the site's user name and password. This information is required to assure that mechanisms usually triggered by web registration (e.g., drug shipment, confirmation of registration, and patient-specific calendar) will occur.

6.0 RADIATION THERAPY
Not applicable to this study.

7.0 DRUG THERAPY
Not applicable to this study.

8.0 SURGERY
Not applicable to this study.

9.0 OTHER THERAPY
No supportive care in excess of that required for routine radiation therapy and chemotherapy should be required.

10.0 TISSUE/SPECIMEN SUBMISSION

10.1 Overall Trial Design

Patients receiving radiotherapy for GBM on another RTOG study will be potential candidates for enrollment on this protocol. This protocol is not a study of an experimental therapy and will be carried out in conjunction with standard radiotherapy for patients receiving radiation as part of a primary treatment of GBM.

Urine samples will be collected at baseline, at the completion of radiation (6000 cGy), and at the 1-month follow-up evaluation.

Urine samples will be evaluated for VEGF and MMP levels and correlated to the disease-free recurrence at 1 year.

10.2 Urine Collection Guidelines

Nurses at the radiation oncology clinic will collect urine samples before, during, and following radiation therapy. (See collection schedule in Section 10.3) Collection guidelines at each time point are the following: **[NOTE: A specimen transmittal form (ST) must accompany each specimen.]**

10.2.1 At least 5 cc of urine in a standard clinical sterile collection cup labeled with patient ID, date and timepoint and stored at range -20°C to 4°C. Each sample may be forwarded when it is collected.

10.2.2 Send urine samples in an overnight FEDEX box covered with dry ice to Dr. Camphausen's laboratory for appropriate processing and storage. Please call 301-496-5457 and ask for the clinic nurse or email camphauk@mail.nih.gov to obtain a FEDEX shipping code. All shipping charges will be paid by the principle investigator. Please ship to:

**Kevin Camphausen
Attn: Clinic Nurse
National Cancer Institute
10 Center Drive
Building 10, CRC Rm B2-3561
Bethesda, MD 20892-1682**

10.2.3 From there, Dr. Camphausen's laboratory will send in batches aliquots of urine to the laboratory of Marsha Moses, Children's Hospital, Harvard Medical School.

10.3 Urine Collection Schedule

Urine will be collected at the following time points, per the collection guidelines listed in Section 10.2:

10.3.1 Pre-treatment (baseline)

10.3.2 On the last day of treatment

10.3.3 Post active treatment evaluation (follow-up): At 1 month following the completion of radiotherapy (after 6000 cGy)

10.4 Urine Analysis

Urine samples will be tested using a commercial Elisa system for VEGF, (R&D systems). Urine samples will also have creatinine levels measured by the clinical pathology department for standardization of the VEGF levels in the urine.

Urine will be evaluated by the laboratory of Marsha Moses for MMP activity using gel zymography. These results will be evaluated by two observers blinded to the clinical profile of the patient who supplied the sample. A binary evaluation of low-MW MMP, MMP-2, MMP-9 and high MW-MMP will be made. Each of the five MMPs will be scored as absent (0) or present (1). The five values for each MMP will be cumulated for each patient to create an MMP score from 0-5.

Urine samples collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document. No germline mutation testing will be performed on any of the samples collected unless the patient gives separate informed consent.

10.5 Reimbursement and Case Credit (5/25/07)

Institutions will be reimbursed \$50 per urine sample, for a total of \$150. Dr. Camphausen's laboratory must have received all three samples in order for institutions to receive any reimbursement, with the exception of patient death. In the event of patient death before all samples have been collected, institutions will receive reimbursement on a pro-rated basis. After confirmation from Dr. Camphausen's laboratory that appropriate materials have been received, RTOG Administration will prepare the proper paperwork and send a check to the institution. Pathology payment cycles are run twice a year in January and July and will appear on the institution's summary report with the institution's regular case reimbursement.

Institutions will receive .5 credit and a \$1000 reimbursement if Dr. Camphausen's laboratory has received all three urine samples and they are sufficient quality that the tumor markers can be determined. These samples will be designated as "usable." In the event of patient death before all samples have been collected, institutions that have submitted at least one urine sample will receive the .5 case credit.

11.0 PATIENT ASSESSMENTS

Not applicable to this protocol.

12.0 DATA COLLECTION

Data should be submitted to:

**RTOG Headquarters
1818 Market Street, Suite 1600
Philadelphia, PA 19103**

Patients will be identified by initials only (first middle last); if there is no middle initial, a hyphen will be used (first-last). Last names with apostrophes will be identified by the first letter of the last name.

12.1 Summary of Data Submission (5/4/06)

<u>Item</u>	<u>Due</u>
Initial Evaluation Form (I1)	Within 2 weeks of study registration
Treatment Summary Form (T1)	Within 2 weeks of completion of radiotherapy
Follow-Up Form (F1)	At 6 and 12 months following study registration

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Endpoint

The primary objective of this study is to evaluate the diagnostic accuracy of a positive increase in either urinary VEGF or MMP from the last treatment measurement (after the 6000 cGy radiation treatment as described in Section 10.3) to the 1-month post radiation treatment follow-up in predicting 1-year recurrence among patients with GBM.

13.2 Sample Size (5/25/07)

Sample size was calculated to get reasonably precise estimates of sensitivity and specificity for 1-year recurrence. We will assume that the 1-year recurrence rate is 50% (averaged over PRA class). If the true sensitivity and specificity of VEGF and MMP increase are both 80% (this is a reasonable assumption based on our previous results summarized in Table 1), the expected 95% confidence interval for estimated sensitivity and specificity would be (0.69, 0.91) with 100 patients. If the true sensitivity (specificity) is 90%, the expected confidence interval for sensitivity (specificity) will be (0.82, 0.98) with 100 patients. The urine specimen data with respect to the submission and quality was evaluated in the first 84 patient entries. These patients had potentially been on study sufficiently long enough for all three specimens to have been collected. All three usable specimens had been received for only 47 (54%) patients. Since the rate of patients with unusable specimens was higher than expected (30% vs 46%), the targeted sample size will be increased to 200 patients, assuming conservatively that all three usable specimens will be received for half of future patients. Once all three usable specimens have been received for 100 patients, the protocol will be closed to new patients even if less than 200 patients have been enrolled.

13.3 Inclusion of Women and Minorities (5/25/07)

The projected gender and ethnicity accruals based upon previous RTOG studies of GBM appear below:

Ethnic Category	Females	Males	Total
Hispanic or Latino	5	9	14
Not Hispanic or Latino	85	101	186
Ethnic Category: Total	90	110	200
Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	0	1	1
Black or African American	11	12	23
Native Hawaiian or other Pacific Islander	2	1	3
White	77	96	173
Racial Category: Total	90	110	200

13.4 Patient Accrual

As this trial is open to all patients on RTOG GBM treatment studies and the accrual to such studies is approximately 20 cases per month, we anticipate that this trial will accrue an average of five cases per month. Allowing for low accrual during the first 6 months while institutions are obtaining institutional review board (IRB) approval, accrual should be completed within 30 months of study activation. If the average monthly accrual rate (excluding the first 6 months) is less than two patients, the study will be re-evaluated with respect to feasibility.

13.5 Analysis plan

13.5.1 Interim Analyses

Interim reports are prepared every 6 months until the initial report of the treatment results has been presented to the scientific community. These reports will contain:

- a) the patient accrual rate with a projected completion date for the accrual phase;
- b) accrual by institution;
- c) distributions of patient characteristics.

Through examining the above items, the statistician and study chair can identify problems with the execution of the study. These problems will be reported to the RTOG Brain Committee

and, if necessary, the RTOG Research Strategy Committee, so that corrective action can be taken.

13.5.2 Final Analysis

Diagnostic accuracy will be assessed by estimating the sensitivity and specificity of an increasing trend (i.e., change dichotomized as either positive or non-positive) in predicting 1-year recurrence. Estimated sensitivity and specificity will be presented for both VEGF and MMP collected from urine. We will estimate other measures of diagnostic accuracy such as positive predictive value and the positive likelihood ratio. Using logistic regression, we will also examine whether the magnitude of the change in VEGF is predictive of 1-year recurrence (i.e., does the probability of a recurrence increase as the change increases?).

We are also evaluating whether changes in VEGF and MMP (from the last treatment to the 1-month post-radiation treatment measurements) add to other established prognostic variables (e.g., RPA class as described in Section 1.4) in predicting 1-year recurrence among patients with GBM. This will be done using logistic regression where 1-year recurrence will be the dichotomize dependent variable and RPA class as well as VEGF/MMP increase will be independent variables. In addition, we will use the Cox proportional hazards model to examine the effect of VEGF/MMP change on the time to recurrence after adjusting for RPA class. We will test for the added predictive effect of VEGF and MMP increase by testing for these effects within the two classes of models; these tests will be done with two-sided likelihood-ratio tests conducted at the 0.05 significance level.

We will investigate whether VEGF and MMP along with RPA class can be used to build a multivariate predictor of 1-year recurrence. This will be done by fitting logistic regression to 1/3 of the data (chosen at random) and using the estimated parameters to fit a ROC curve to the remaining 2/3 of the data. We will repeat this analysis 1,000 times (e.g., cross-validation), and the ROC curves will be averaged. The added predictive ability of VEGF/MMP trending will be assessed by comparing the average ROC curve obtained with VEGF/MMP included with the average curve obtained with VEGF/MMP excluded.

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly by electronic means. Reports are due January 31, April 30, July 31, and October 31.

REFERENCES

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*, 285: 1182-1186, 1971.
2. Smith BD, Smith GL, Carter D, et al. Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. *J Clin Oncol*, 18: 2046-2052, 2000.
3. Linderholm B, Tavelin B, Grankvist K, Henriksson R. Vascular endothelial growth factor is of high prognostic value in node-negative breast carcinoma. *J Clin Oncol*, 16: 3121-3128, 1998.
4. Kraft A, Weindel K, Ochs A, et al. Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. *Cancer*, 85: 178-187, 1999.
5. Verheul HM, Hoekman K, Jorna AS, et al. Targeting vascular endothelial growth factor blockade: ascites and pleural effusion formation. *Oncologist*, 5: 45-50, 2000.
6. Kaban K, Herbst RS. Angiogenesis as a target for cancer therapy. *Hematol Oncol Clin North Am*, 16: 1125, 2002.
7. Ondruschka C, Buhtz P, Motsch C, et al. Prognostic value of MMP-2, -9 and TIMP-1, -2 immunoreactive protein at the invasive front in advanced head and neck squamous cell carcinomas. *Pathol Res Pract*, 198: 509-515, 2002.
8. Cai W, Song JD. Expression of matrix metalloproteinase and tissue inhibitors in ovarian tumors. *Ai Zheng*, 21: 91-94, 2002.
9. Lengyel E, Schmalfeldt B, Konik E, et al. Expression of latent matrix metalloproteinase 9 predicts survival in advanced ovarian cancer. *Gynecol Oncol*, 82: 291-298, 2001.
10. Sielens W, Hellers J, Morresi-Hauf A, et al. Prognostic impact of matrix metalloproteinase-9 in operable non-small cell lung cancer. *Int J Cancer*, 103: 647-651, 2003.
11. Kuitinen O, Soini Y, Turpeenniemi-Hujanen T. Diverse role of MMP-2 and MMP-9 in the clinicopathological behavior of Hodgkin's lymphoma. *Eur J Haematol*, 69: 205-212, 2002.
12. Gerhards S, Jung K, Koenig F, et al. Excretion of matrix metalloproteinases 2 and 9 in urine is associated with a high stage and grade of bladder carcinoma. *Urology*, 57: 675-679, 2001.
13. Kausch I, Bohle A. Molecular aspects of bladder cancer III: prognostic markers of bladder cancer. *Eur Urol*, 41: 15-29, 2002.
14. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. *J Clin Oncol*, 19: 1207-1225, 2001.
15. Moses MA, Wiederschain D, Loughlin KR, et al. Increased incidence of matrix metalloproteinases in urine of cancer patients. *Cancer Res*, 58: 1395-1399, 1998.
16. Mayerhofer M, Valent P, Sperr WR, et al. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1a, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. *Blood*, 100: 3767-3775, 2002.
17. Dvorak HF, Nagy JA, Berse B, et al. Vascular permeability factor, fibrin, and the pathogenesis of the tumor stroma formation. *Ann N Y Acad Sci*, 667: 110-111, 1992.
18. Vihinen P, Kahari VM. Matrix metalloproteinases in cancer: Prognostic markers and therapeutic targets. *Int J Cancer*, 99: 157-166, 2002.
19. Burke PA, DeNardo SJ. Antiangiogenic agents and their promising potential in combined therapy. *Crit Rev Oncol Hematol*, 39: 155-171, 2001.
20. Curran W, Scott C, Horton J, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst*, 85: 704-710, 1993.
21. Jeremic B, Milicic B, Grujicic D, et al. Multivariate analysis of clinical prognostic factors in patients with glioblastoma multiforme treated with a combined modality approach. *J Cancer Res Clin Oncol*, 129: 477-484, 2003.
22. Tortosa A, Vinolas N, Villa S, et al. Prognostic implication of clinical, radiologic, and pathologic features in patients with anaplastic gliomas. *Cancer*, 97: 1063-1071, 2003.
23. Akman F, Cooper R, Sen M, et al. Validation of the Medical Research Council and a newly developed prognostic index in patients with malignant glioma: how useful are prognostic indices in routine clinical practice? *J Neurooncol*, 59: 39-47, 2002.
24. Lacroix M, Abi-Said D, Fourny D, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg*, 95: 190-198, 2001.
25. Scott J, Rewcastle N, Brasher P, et al. Long-term glioblastoma multiforme survivors: a population-based study. *Can J Neurol Sci*, 25: 197-201, 1998.
26. Thiessen B, Maguire J, McNeil K, et al. Loss of heterozygosity for loci on chromosome arms 1p and 10q in oligodendroglial tumors: relationship to outcome and chemosensitivity. *J Neurooncol*, 64: 271-278, 2003.

27. Pollack I, Finkelstein S, Burnham J, et al. Association between chromosome 1p and 19q loss and outcome in pediatric malignant gliomas: results from the CCG-945 cohort. *Pediatr Neurosurg*, 39: 114-121, 2003.
28. Hashimoto N, Murakami M, Takahashi Y, et al. Correlation between genetic alteration and long-term clinical outcome of patients with oligodendroglial tumors, with identification of a consistent region of deletion on chromosome arm 1p. *Cancer*, 97: 2254-2261, 2003.
29. Tchirkov A, Rolhion C, Kemeny J, et al. Clinical implications of quantitative real-time RT-PCR analysis of hTERT gene expression in human gliomas. *Br J Cancer*, 88: 516-520, 2003.
30. Chan L, Moses M, Goley E, et al. Urinary VEGF and MMP levels as predictive markers of 1-year progression-free survival in cancer patients treated with radiation therapy: a longitudinal study of protein kinetics throughout tumor progression and therapy. *J Clin Oncol*, 22: 499-506, 2004.

APPENDIX I

RTOG 0611 (5/4/06, 5/25/07)

Informed Consent Template for Cancer Treatment Trials **(English Language)**

URINARY VEGF AND MMP LEVELS IN PATIENTS RECEIVING RADIATION THERAPY FOR GLIOBLASTOMA MULTIFORME: PROSPECTIVE DETERMINATION OF A PREDICTIVE VALUE FOR RECURRENCE

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

You are being asked to take part in this study because you have a brain tumor that is classified as a glioblastoma multiforme and you are being treated with radiation therapy for your tumor as part of another RTOG study. The investigators of RTOG study 0611 would like to analyze samples of your urine to see whether the protein contents of your samples can help tell them about your response to the radiation.

Why is this study being done?

The purpose of this study is to try find out if samples of urine taken before, during, and after radiation therapy for glioblastoma multiforme reveal information about how patients respond to radiation for this disease.

How many people will take part in the study? (5/25/07)

About 200 people will take part in this study.

What will happen if I take part in this research study?

We would like to keep some of your left over urine to use for research. If you agree to participate in this study, small samples of your urine will be collected and shipped to researchers who work at pathology laboratories at the National Cancer Institute and Harvard University, so that the researchers can analyze the protein make-up of these samples.

You would be asked to give samples at the following times:

- Before you receive radiation therapy as part of your treatment on another RTOG study, and
- On the last day of your radiation therapy
- 1 month after you have finished radiation therapy

You would give these samples during routine visits to your doctor as part of your participation on the other RTOG study.

Your urine will not be used for research about other diseases (to learn about, prevent, or treat other health problems such as other cancers, diabetes, Alzheimer's disease, or heart disease) unless you give your permission.

How long will I be in the study?

Your time spent giving urine samples will span the course of about 3 months (from just before you start radiation therapy to 1 month after you complete the therapy). Your urine will be kept indefinitely unless you request that it be disposed of.

Can I stop being in the study?

Yes. You can decide to stop participating at any time. If you decide now that your urine can be kept for research, you can change your mind at any time. If you change your mind, contact your study doctor and let him/her know.

What side effects or risks can I expect from being in the study?

If you agree to participate in this study, you may be asked to give more urine than you would normally be asked to give as part of your treatment for glioblastoma multiforme.

The only risk you would face is the possible release of information from your health records. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone else is very small. Giving urine for these research purposes will not add any additional risks to your current treatment for your tumor.

Are there benefits to taking part in the study?

The benefits of research using your urine include learning more about what causes glioblastoma multiforme and other brain tumors, how to prevent them, and how to treat them. You will not benefit directly from the research.

What other choices do I have if I do not take part in this study? (5/25/07)

Your other choices may include:

- Taking part in another study

Talk to your study doctor about your choices before you decide if you will take part in this study.

Will my medical information be kept private? (5/25/07)

Data are housed at RTOG Headquarters in a password-protected database. We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The Radiation Therapy Oncology Group
- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people

What are the costs of taking part in this study?

Your permission to collect, store, and analyze your urine will not result in additional cost to you or your insurance company.

You will not be paid for taking part in this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at <http://cancer.gov/clinicaltrials/understanding/insurance-coverage>. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ [*investigator's name(s)*], if you feel that you have been injured because of taking part in this study. You can tell the study doctor in person or call him/her at _____ [*telephone number*].

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ [*name(s)*] at _____ [*telephone number*].

For questions about your rights while taking part in this study, call the _____ [*name of center*] Institutional Review Board (a group of people who review the research to protect your rights) at _____ (*telephone number*).

Making Your Choice

Please read each sentence below and think about your choice. After reading each sentence, circle "Yes" or "No". If you have any questions, please talk to your study doctor or nurse, or call our research review board at IRB's phone number.

No matter what you decide to do, it will not affect your care.

1. My urine may be kept for use in this research study to learn about, prevent, or treat brain tumors.

Yes No

2. My urine may be kept for use in research to learn about, prevent, or treat other cancers.

Yes No

3. My urine may be kept for use in research to learn about, prevent or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease).

Yes No

4. Someone may contact me in the future to ask me to take part in more research.

Yes No

Where can I get more information?

You may call the National Cancer Institute's Cancer Information Service at:

1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

You may also visit the NCI Web site at <http://cancer.gov/>

- For NCI's clinical trials information, go to: <http://cancer.gov/clinicaltrials/>
- For NCI's general information about cancer, go to <http://cancer.gov/cancerinfo/>

You will get a copy of this form. If you want more information about this study, ask your study doctor.

Signature

I have been given a copy of all _____ *[insert total of number of pages]* pages of this form. I have read it or it has been read to me. I understand the information and have had my questions answered. I agree to take part in this study.

Participant _____

Date _____