RADIATION THERAPY ONCOLOGY GROUP

RTOG 95-05

EVALUATION OF TUMOR ANGIOGENESIS MEASURED WITH MICROVESSEL DENSITY *(MVD)* AS A PROGNOSTIC INDICATOR IN NASOPHARYNGEAL CARCINOMA

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1.0 INTRODUCTION (9/8/98)

Tumor growth and metastasis are angiogenesis dependent.[4,7,10,24,39] Human retinoblastomas, metastatic to the vistrous of the anterior chamber are avascular and growth restricted although they remain viable. Carcinoma of the ovary metastasizes to the peritoneal membrane as tiny avascular seeds, which do not grow beyond a limited size without neovascularization.[10] Inhibitors of angiogenesis, such as angioinhibin *(also known as AGM-1470 or TNP 470)* which are not cytostatic to tumor cells in vitro inhibit tumor growth in vivo.[9,18] Inhibition of vascular endothelial growth factor *(VEGF)* induced angiogenesis suppresses tumor growth in vivo.[20] For a tumor to metastasize, tumor cells must gain access to the vasculature from the primary tumor, survive the circulation, localize the microvasculature of the target organ, escape from *(or grow from within)* the vasculature into the target organ and induce angiogenesis in the target organ. Thus, angiogenesis is necessary in the initiation of the metastatic process as well as establishing growth in the target organ.

The mechanism of tumor angiogenesis is not fully understood. Neovascularization is mediated by specific angiogenic molecules released by the tumor cells and/or host immune cells into the tumor stroma.[4,11,26] Weidner, et al.[38] noted that some breast ducts containing ductal carcinoma-in-situ *(DCIS)* may have a ring of neovascularization around the duct. The close proximity of this ring of neovascularization to the DCIS cells, suggested that it was formed in response to angiogenic factor*(s)* released by the DCIS cells. Cytokines secreted by activated macrophages such as transforming growth factor- alpha *(TGFa, angiotropin, tumor necrosis factor-alpha (TGFa)* and basic fibroblast growth factor *(bFGF)* have angiogenic properties. [1,4,11-13,16,22,26,29] These cytokines secreted by host immune cells as well as the angiogenic factors secreted by the tumor cells may play a role in tumor neovascularization.

Since tumor angiogenesis is necessary for the initiation as well as establishment of metastasis, the intensity of angiogenesis may predict the probability of metastasis. Indeed, in cutaneous melanoma, Srivastava and his colleagues showed that there was a sharp break point between a stage of relative absence of neovascularization, which correlated with absence of metastasis and a stage in which increased neovascularization correlated with an increased metastatic rate.[32] Furthermore, in patients with intermediate-thickness cutaneous melanomas, those who developed metastases showed a vascular area at the tumor base that was more than twice that in patients without metastases. The age, sex, Breslow's tumor thickness, and Clark's level of invasion were similar in the two groups.[32]

In 1991, Weidner and his colleagues first showed a statistically significant correlation between incidence of metastasis and microvessel density in a group of 49 unselected patients with primary invasive breast carcinoma.[38] In this study, all microvessels in the tumor specimens were highlighted by immunoperoxidase staining of endothelial cells for factor VIII-related antigen *(F8-RA).* Hematoxylin and Eosin *(H & E)* stained sections of the tumor were used to select areas representative of the invasive component. MVD was scored by light microscopy in areas of invasive tumor containing the highest numbers of capillaries and small venules *(microvessels)* per 200 X field. These high neovascular areas could occur anywhere within the tumor, but most frequently appeared at the margins of the invasive carcinoma. The incidence of metastasis increased with MVD, reaching 100% for patients having MVD of > 100 per 200 X field. The mean MVD was 101 per 200 X field *(SD=49.3, range, 16-220)* in patients with metastases and 45 per 200 X field *(SD=21.1 range, 15-100)* in patients without metastases *(p=0.003)*. Multivariate analysis showed MVD to be the best predictor of metastasis when compared to tumor grade and tumor size. Subsequent studies showed that MVD in the area of the most intense neovascularization in invasive breast carcinoma was an independent and highly significant prognostic indicator for overall and relapse-free survival in patients with early-stage and node-negative breast carcinoma.[5,14,17,28,31, 33,35,37] These findings suggest a role for MVD in the selection of high-risk node-negative breast carcinoma patients for adjuvant therapies.

However, there are also studies that showed no significant correlation between MVD and prognosis in breast carcinomas.[15,34] Although the procedure of selecting areas for microvessel counting in these studies differed from that used in the studies by Weidner et al. and the median follow-up was also shorter, being only 1.5 years in the study by Hall et al.

In addition to breast carcinomas the association between MVD and prognosis has also been shown in non-small cell lung cancer[23] prostate cancer [2,3,36] and head and neck cancer.[14]

Among all head and neck carcinomas, nasopharyngeal carcinoma has the highest incidence of distant metastasis. Although local-regional control of nasopharyngeal carcinoma by radiotherapy has improved with modern imaging techniques and radiotherapy treatment planning and delivery,[25] overall survival has not changed and remains about 50% at five years in most series. The incidence of distant metastasis is in the range of 20-60%. Non-randomized studies suggest improved survival with the use of adjuvant chemotherapy. However, one randomized trial of adjuvant chemotherapy after radiotherapy for nasopharyngeal carcinoma was negative.[27] Recently, several randomized trials in head and neck cancers suggest a decreased incidence of distant metastasis with neoadjuvant or adjuvant chemotherapy.[8,19,21,30] Randomized studies comparing radiotherapy and adjuvant chemotherapy to radiotherapy alone for nasopharyngeal carcinoma are in progress in the United States, Asia, Europe and Africa.[6] However, chemotherapy, like radiotherapy, has undesirable side effects and potential complications and not all patients will benefit from chemotherapy. Thus, there is a need for predictors of metastatic potential and prognosis. These predictors may be useful in the selection of patients for systemic therapy or anti-angiogenesis drugs in future trials.

Preliminary results of a retrospective study of 30 patients with nasopharyngeal carcinoma treated at the University of California, San Francisco showed a significant correlation between microvessel count per 200 x field and the incidence of distant metastasis, disease-free-survival and overall survival. The incidence of distant metastases was 62.5% vs. 17% *($p = 0.026$)*, 5-yr. disease-free survival was 10% vs. 28% *($p = 0.05$)* and overall survival was 13% vs. 50% *($p = 0.027$)* for patients with microvessel count ≥ 60 or < 60 respectively.[40] These results suggest that microvessel density may be an important prognostic factor in nasopharyngeal carcinoma and warrant further confirmation with larger numbers of patients.

In this study, MVD will be determined in the pre-treatment biopsy specimens of patients with nasopharyngeal carcinoma, treated with radiotherapy alone or in combination with a radiosensitizer in RTOG protocols 79-13, 79-15, 83-13, and 85-27. RTOG 79-15 and RTOG 85-27, phase III studies testing misonidazole and SR-2508 respectively, showed no significant improvement with the addition of three sensitizers. Non RTOG protocol patients with primary squamous cell carcinoma of the nasopharynx treated with radiotherapy alone in RTOG member institutions between 1980 and 1990 with adequate follow-up information available will also be eligible. MVD will be correlated with incidence of distant metastasis, local-regional control, disease-free survival and overall survival. The results should establish the prognostic significance of tumor angiogenesis measured by MVD in nasopharyngeal carcinoma that in the future may aid in the selection of treatment for the individual patient.

The Southwest Oncology Group *(SWOG)* coordinated the Intergroup study *(INT #0099)* with the participation of Radiation Therapy Oncology Group (RTOG), and Eastern Cooperative Oncology Group *(ECOG).* This randomized phase III trial compared chemoradiotherapy versus radiotherapy alone in patients with nasopharyngeal cancers. It has been completed and the results published. Chemoradiotherapy has found to be superior to radiotherapy alone for patients with advanced nasopharyngeal cancers with respect to progression-free survival and overall survival.[47] Because it has become the standard treatment, this laboratory correlative study has been modified to include the patients treated on this chemoradiotherapy program. If prognostic significance of MVD is confirmed in patients treated on radiation alone, then its significance can be evaluated in patients treated on the combined modality approach.

2.0 OBJECTIVES (9/8/98)

2.1 To evaluate MVD as an independent prognostic factor in patients with nasopharyngeal carcinoma *(NPC)* and treated with radiation therapy alone by correlating MVD with incidence of distant metastasis, local-regional control, disease-free survival and overall survival.

2.2 To evaluate MVD as an independent prognostic factor in patients with nasopharyngeal carcinoma (NPC) who have been treated with radiation therapy and chemotherapy according to the intergroup trial INT 0099/RTOG 88-17 by correlating MVD with incidence of distant metastasis, local-regional control, disease-free survival and overall survival.

2.3 To correlate MVD with WHO histopathological type of nasopharyngeal carcinoma.

3.0 PATIENT SELECTION (9/8/98)

3.1 Patients with squamous cell carcinoma of the nasopharynx treated with radiotherapy ± radiosensitizer *(misonidazole or SR-2508)* in RTOG's protocols 79-13, 79-15, 83-13, and 85-27 will be eligible.

3.2 Non-RTOG protocol patients with primary squamous cell carcinoma of the nasopharynx treated with radiotherapy alone between 1975 and 1996 with a minimum of two years of followup information available will also be eligible. Slides and blocks must be available.

3.3 Patients with a diagnosis of squamous cell carcinoma of the nasopharynx whose initial treatment plan included chemotherapy which was administered according to the combined modality arm of intergroup nasopharynx study INT 0099, *(see Section 7.0),* are eligible provided that there has been compliance with all of the following:

- Patients must have received their initial treatment with this regimen between January 1,1990 and December 31, 1996.
- Patients need not have completed all planned chemotherapy courses, however, a minimum of one full dose of the eligible chemotherapy regimen administered with concurrent radiotherapy must have been given for the patient to be eligible for this study.
- Surviving patients treated with the eligible chemotherapy regimen must be available for yearly followup.
- The required slides/blocks with tumor can be submitted as specified in Section 10.0 and all other criteria have been met.

3.4 Patients who expired within two years of initial treatment are eligible, provided adequate information is available.

3.4.1 The following information must be available on each patient: initial TNM Classification, details and dates of the initial treatment regimen, disease status at two years or at death *(if survival is less than 2 years)*, slides and blocks *(see Section 10.0)* of the tumor specimen obtained at *diagnosis (prior to commencement of any treatment*).

3.5 Study-specific informed consent must be obtained from living patients.

4.0 PRETREATMENT EVALUATIONS

Not applicable.

5.0 REGISTRATION (9/8/98)

5.1 Patients can be registered only after eligibility criteria are met. Patients are registered by calling RTOG headquarters at *(215)* 574-3191, Monday through Friday 8:30 am to 5:00 pm ET. The patient will be registered and a case number will be assigned and confirmed by mail. The following information must be provided:

- Institution Name & Number
- Patient's Name & ID Number
- Verifying Physician's Name
- Eligibility Criteria Information
- Demographic Data
- Stratification

5.2 Option Assignments

5.2.1 Patients previously treated with radiation only will be assigned to Option 1.

5.2.2 Patients who were not treated on an RTOG study but were treated with radiation only will be assigned to Option 2.

5.2.3 Patients who were not treated on an RTOG-coordinated study but were treated with radiation therapy and chemotherapy according to the SWOG-coordinated intergroup study INT 0099/ RTOG 88-17 will be assigned to Option 3.

6.0 RADIATION THERAPY (9/8/98)

6.1 Patients will have completed radiotherapy ± radiosensitizer as entered onto RTOG protocols 79-13, 79-15, 83-13, and 85-27.

6.2 Non RTOG protocol patients will have completed radiotherapy alone at RTOG member institutions between 1975 and 1996.

6.3 Non RTOG protocol patients will have completed radiotherapy portion of the combined modality program at RTOG member institutions between 1990 and 1996.

7.0 DRUG THERAPY

7.1 Chemotherapy administered with initial radiotherapy *(between 1990 and 1996)* is restricted to the following regimen:

Concurrent Chemotherapy and Radiation Therapy

Cisplatinum 100 mg/m^2 IV administered on days 1, 22, and 43. Each course to be administered concurrent with the radiation therapy regimen for a maximum of 3 courses of drug.

Following Completion of Radiation Therapy

Beginning on day 71, administer Cisplatinum 80 mg/m^2 IV and 5-FU 1000 mg/m^2 /day IV for 4 days *(total of 5-FU 4000 mg/m^2 over 96 hours).* The combination is repeated on day 99 and on day 127 for a total of 3 courses.

7.2 Chemotherapy administered for recurrence following tumor relapse is at the discretion of the investigator

8.0 SURGERY

Not applicable.

9.0 OTHER THERAPY

Not applicable.

10.0 PATHOLOGY (11/15/95, 9/8/98)

10.1 Institutional Preparation of Tumor Sections

10.1.1 Paraffin blocks of the primary tumor will have an H and E stained section prepared of the block face. These H and E stained sections will be used to choose areas representative of the invasive component. One representative section per tumor will be stained for factor VIII-related antigen *(F8-RA)* using a standard immunoperoxidase staining technique as described previously.[38]

10.1.2 When only unstained slides but not paraffin block blocks are available, these unstained slides *(5-10)* will be submitted to LDS Hospital for forwarding to Dr. Weidner. Dr. Weidner will stain these slides for factor VIII-related antigen *(F8-RA)* using the immunoperoxidase staining technique. The LDS Hospital address is: LDS Hospital, Dept. of Pathology, E.M. Laboratory, 8th Avenue & C Street, Salt Lake City, UT 84143.

10.1.3 To help ensure that an adequate tissue sample is available for analysis, the biopsy material submitted must have been obtained preferably from the primary site by incisional or excisional biopsy, not cytology. If the reference pathologist finds the material submitted is inadequate for analysis, the case will be considered ineligible retrospectively.

10.2 Procedures Followed by the Reference Pathologist

10.2.1 Immunoperoxidase Staining for Factor VIII-related Antigen.

10.2.2 Microvessel densities *(MVD)* scoring.

10.2.3 Microvessel density will be determined by light microscopy in areas of invasive tumor containing the highest numbers of capillaries and small veniels *(microvessels)* per area *(i.e. areas of the most intense neovascularization).*

10.2.4 Tumor sections will be first scanned at a low power *(40 x and 100 x)* to identify areas of invasive carcinoma having the greatest numbers of distinct factor VIII-related antigen staining microvessels per area *(brown staining)*, usually at the margins of the carcinoma.

10.2.5 Individual microvessel counts will then be made on a 200 x field *(20 x objective and 10 x ocular; equivalent to 0.7386 mm^2 per 200 x field)* within the area of most intense tumor neovascular.

10.2.6 Any endothelial cell or endothelial cell cluster, positive for factor VIII-related antigen and clearly separate from an adjacent cluster will be considered to be a single countable microvessel.

10.2.7 Results will be expressed as the highest number of microvessels identified within any single 200 x field.

10.2.8 The microvessel density scoring and the WHO histopathological classification will be performed by Dr. Noel Weidner in the Department of Pathology of the University of California, San Francisco. His review will be done without any knowledge of the clinical outcome. The results of his review will be forwarded to RTOG Headquarters for correlation with clinical outcome.

11.0 PATIENT ASSESSMENTS

Not applicable.

12.0 DATA COLLECTION (9/8/98)

12.1 Material Due

Item	Due
Pathology Report **(P1)**	Within 3 weeks of study registration
Blocks & Slides **(P2)**	
Patient Status Report **(VS)** *(Options 2 &3)*	
Chemo Summary Form **(F4)** *(Option 3)*	
Yearly Follow-up Form **(FF)** *(Option 3)*	Yearly post study registration

12.2 Paraffin blocks or 5-10 unstained pathology slides of pre-treatment biopsies of the primary tumor **will be sent to LDS Hospital** and must be accompanied by a Pathology Submission Form *(Appendix II)*. In RTOG non-protocol patients, the Patient Status Report *(Appendix IV)* will be submitted as well. The Pathology Report*(s)* for the submitted samples must also be included.

- Paraffin blocks or 5-10 unstained pathology slides of the pre-treatment biopsies of the primary tumor.
- Pathology reports.
- Pathology Submission Form *(Appendix II)*. A separate Submission Form must be used for each patient and must accompany the above. Include the study and case number of the prior treatment protocol.
- Send to:

LDS Hospital
Dept. of Pathology
E.M. Laboratory
8th Ave & C Street
Salt Lake City, UT 84143

13.0 STATISTICAL CONSIDERATIONS (9/8/98)

13.1 Background

A pilot study of microvessel density *(MVD)* in patients with nasopharyngeal tumors was done at UCSF. It could not be determined in two of the 30 patients with blocks. The remaining 28 patients were divided into two groups by MVD values *(< 60 versus ≥ 60)*. Twenty patients *(71%)* had the lower values and eight patients *(29%)* had the higher values. The patients with the higher values did significantly poorer in terms of distant metastases *($p < .01$)*, disease free survival *($p = .014$)*, and overall survival *($p = .0277$)*. This correlative study will try to confirm the pilot results that tumor angiogenesis measured by MVD is predictive of metastatic potential which leads to shorter disease free survival and overall survival. It will also look at local regional control and examine whether there is correlation between MVD and histopathological types.

The study will now be considered as having two parts with separate analyses. The first part will be restricted to patients treated with radiotherapy alone or in combination with a radiosensitizer *(misonidazole or SR 2508)*. These were shown to be ineffective in RTOG studies. The second part will be restricted to patients treated with radiation therapy and chemotherapy according to the intergroup trial INT 0099/RTOG 88-17. This program was shown to significantly improve survival in the intergroup trial.

Because the intergroup trial INT 0099 has established chemoradiation as the standard treatment, this laboratory correlative study has been modified to include the patients treated with this regimen. If prognostic significance of MVD is confirmed in patients treated on radiation alone, then its significance can be evaluated in patients treated on the combined modality approach.

13.2 Study Population

From the most recent analyses of the data from RTOG 79-13, 79-15, 83-13, and 85-27, it has been determined that treatment outcome information is available in a total of 180 patients with nasopharyngeal carcinoma treated with radiotherapy ± radiosensitizer. The breakdown of the number of patients by protocol is as follows:

Study	Treatment Option	# Nasopharynx pts
79-13	RX 1 *(RT)*	6
	RX 2 *(RT)*	9
79-15	RX 1 *(RT)*	16
	RX 2 *(RT &Misonidazole)*	16
83-13	RX 1-4 (RT)	51
Study	Treatment Option	# Nasopharynx pts
85-27	RX 1 *(RT + SR2058)*	4
	RX 2 *(RT + SR2058)*	36
	RX 3 *(RT)*	42
TOTAL		180

It is realized that biopsy specimens will not be available for all patients targeted in these studies. On the other hand, additional eligible patients not entered on RTOG protocols may have biopsy specimens available for this study. However, there is little concern over selective availability of these samples. That is, it is not any more likely to obtain samples for patients who developed metastasis then the patients who had no metastasis. Factors affecting sample availability include, how long ago the samples were taken, the general policies of maintaining patient samples in a given institution and the willingness of each institution to retrieve these samples for this study. Regardless, any analyses performed relating incidence of distant metastasis and treatment outcome to MVD are not likely to be confounded by selection bias.

In addition, the study population has been expanded to include Non-RTOG protocol patients with primary squamous cell carcinoma of the nasopharynx whose treatment plan was the combined modality arm of intergroup nasopharyngeal trial INT 0099. Patients who started RT and received one concurrent Cis-platinum dose are eligible. Patients do not have to complete either radiation therapy or the chemotherapy.

13.3 Sample Size

13.3.1 *Patients Treated with RT Alone*

In the UCSF pilot study, the prevalence of MVD values ≥ 60 was 28.6%. For planning purposes, we will assume that the prevalence is 30%. For completeness, calculations were also done for prevalence rates of 20% and 40%. The survival for patients with high MVD values *(i.e. ≥ 60)* was significantly poorer than for patients with low MVD values *(p= 0.027)* with associated hazard ratio 2.67. We would like to detect a hazard ratio of at least 2.5 with 90% statistical power. Similarly, calculations will be done to detect hazard ratios of 2.0, 3.0, and 3.5. Statistical power was calculated using the equation described by Shoenfeld.[48]

Number failures = $(z_{1-\alpha/2} = z_{1-\beta})^2 / (\ln HR)^2 \, w\,(1 - w)$, where

$z_{1-\alpha/2}$ = normal deviate for the significance level
$z_{1-\beta}$ = normal deviate for the statistical power
HR = hazard ratio
w = prevalence rate for patients with MVD ≥ 60

Setting the statistical significance level at 0.05, the following table represents the number of failures needed to detect hazard ratios of 2.0, 2.5, 3.0, and 3.5, respectively, with 90% statistical power.

Incidence \ HR	2.0	2.5	3.0	3.5
20%	137	79	55	42
30%	105	60	42	32
40%	92	53	37	28

Survival:

Sixty deaths will be required to confirm the survival result from the pilot. If the prevalence rate is as low as 20%, there will still be least 80% statistical power to detect a hazard ratio of 2.5. Setting the number of deaths at 60, the statistical power *(see Table below).*

Incidence	Power
20%	81%
30%	90%
40%	94%

Distant Metastases:

For the endpoint of time to distant metastases, the number of failures was set at 32 occurrences. The statistical power was calculated to detect a hazard ratio of 3.5 for incidence rates of 20%, 30%, and 40%, respectively.

Incidence	Power
20%	81%
30%	90%
40%	93%

Since better than 50% of patients that die have distant metastases, the accrual goal for the part of the study is 60 patients who died and have MVD determinations.

13.3.2 *Patients Treated with Combined Modality Arm from INT 0099*

For patients treated with this combined modality arm, the accrual goal will be the same as that of the RT alone arm, namely 60 patients with MVD determinations who died.

13.4 Analysis Plans

RTOG 85-27 had 83 eligible nasopharynx patients. Of these 83, 40 were treated with RT + sensitizer SR2508 and 43 were treated with RT alone. There was no statistically significant difference in overall survival *(p = 0.67, hazard ratio = 1.13)* or in time to distant metastases *(p = 0.71, hazard ratio = 1.14)* between the two treatment arms. Because the hazard ratio for each endpoint is so close to identity, there will be no adjustment for the administration of SR2508. The non-protocol patients will have received initially radiotherapy using various fractionation schedules and total dose, with no sensitizer. In reviewing the chapter on nasopharynx from Perez and Brady's Principles and Practice of Radiation Oncology,[49] total dose was reported to have an impact on outcome. So total dose will be used in the multivariate analyses

There will be two analyses. First, as soon as 60 patients treated with the RT arm have been accrued who have died and have MVD determination. The second analysis will take place after 60 patients treated with the combined modality have been accrued who have died and have MVD determination.

Both univariate and multivariate analyses of MVD will be performed for all the endpoints of interest. For time to distant metastases, the cumulative incidence approach will be used to estimate it as a function of time because this approach specifically accounts for competing risks such as dying without distant metastases.[42] The distributions of the times to the distant metastases between the two MVD groups *(< 60 versus ≥ 60)* be compared by a method developed.[43] Local regional control will be analyzed in a similar fashion. The disease free and the overall survival rates will be estimated by the Kaplan Meier method and will be compared by the log-rank test.[44]

The multivariate Cox proportional hazard model46 will be employed to examine the relationship between MVD and various disease outcomes while adjusting for other concomitant variables *(e.g. age, sex, T-Stage, N-Stage, Karnofsky performance score, total dose).* All the factors initially will be considered as

dichotomous variables in the model. In determining whether a relationship exists for a particular outcome, tests of significance will be compared to an alpha level that is adjusted for multiple comparisons in order to assure that the overall level of Type I error does not exceed 5%.[45]

REFERENCES

1. Baird, A., Mormede, P., Bohlen, P.: Immunoreactive fibroblast growth factor in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. Biochem Biophys Res Commun 126: p. 358-364, 1985.

2. Bigler, S.A., Brawer, M.K., Deering, R.E.: Neovascularization in carcinoma of the prostate: A quantitative morphometric study. Pathol 5: p. 50a, 1992.

3. Bigler, S.A., Brawer, M.K., Deering, R.E.: Vessel density in carcinoma of the prostate: Comparing organ confined tumors with stage D tumors. Mod Path 6: p. 56a, 1993.

4. Blood, C.H., Zetter, B.R.: Tumor interactions with the vasculature: Angiogenesis and tumor metastasis. Biochimica et Biophysica Acta 1032: p. 89-118, 1990.

5. Bosari, S., Lee, A.K.C., DeLellis, and R.A.: Microvessel quantitation and prognosis in invasive breast carcinoma. Hum Pathol 23: p. 755-761, 1992.

6. Cvitkovic, E., Bachouchi, M., Armand, J.P.: Nasopharyngeal carcinoma. Biology natural history, and therapeutic implications. Hematol Oncol Clin North Am 5(4): p. 821-838, 1991.

7. Fidler, I.J., Gersten, D.M., Hart, I.R.: The biology of cancer invasion and metastasis. Advances in Can Res 28: p. 149-250, 1978.

8. Final Report of the Head and Neck Contracts Program: Cancer 60: p. 301-311, 1987.

9. Folkman, J.: Angiogenesis and its inhibitors. ed. Philadelphia: Lippincott, 1985, pp 42-62

10. Folkman, J.: What is the evidence that tumors are angiogenesis-dependent? Natl Cancer Inst 82: p. 4-6, 1990.

11. Folkman, J., Klagsbrun, M.: Angiogenic factors. Science 235: p. 442-447, 1987.

12. Folkman, J., Klagsbrun, M., Sasse, J.: Heparin-binding angiogenic protein-basic fibroblast growth factor-is stored within basement membrane. Am J Pathol 130: p. 393-400, 1988.

13. Frater-Schroder, M., Risau, W., Hallmann, R.: Tumor necrosis factor type □□ a potent inhibitor of endothelial cell growth in vitro, is angiogenic in vivo. Proc Natl Cad Sci 84: p. 5277-5281, 1987.

14. Gasparini G, Weidner N, Maluta S, Pozza F, Boracchi P, Mezzetti M, Testolin A, Bevilacqua P, Intratumoral microvessel density and p53 protein: correlation with metastasis in head-and-neck squamous cell carcinoma. International Journal of Cancer. 55(5): 739-744, 1993.

15. Hall, N.R., Fish, D.E., Hunt, N.: Is the relationship between angiogenesis and metastasis in breast cancer real? Surg Oncol 1: p. 223-229, 1992.

16. Hockel, M., Jung, W., Vaupel, P.: Purified monocyte derived angiogenic substance (angiotropin) induces controlled angiogenesis associated with regulated tissue proliferation in rabbit skin. J Clin Invest 82: p. 1075-1090, 1988.

17. Horak, E., Leek, R., Klenk, N.: Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet 340: p. 1120-1124, 1992.

18. Ingber, D., Fujita, T., Kishimoto, S.: Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth. Nature 348: p. 555-557, 1990.

19. Jaulerry, C., Rodriguez, J., Brunin, F.: Induction chemotherapy in advanced head and neck tumors: Results of two randomized trials. Int J Radiat Oncol Biol Phys 23: p. 483-489, 1992.

20. Kim, K.J., Li, B., Winer, J. et al.: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo. Nature 362: p. 841-844, 1993.

21. Laramore, G.E., Scott, C.B., Al-Sarraf, M.: Adjuvant chemotherapy for resectable squamous cell carcinomas of the head and neck: Report on intergroup study 0034. Int J Radiat Oncol Biol Phys 23: p. 705-713, 1992.

22. Liebovich, S.J., Plverini, P.J., Shepard, H.M.: Macrophage-induced angiogenesis is mediated by tumor necrosis factor-□. Nature 329: p. 630-632, 1987.

23. Macchiarini, P., Fontaini, G., Hardin, M.J.: Relation of neovasculature to metastasis of non-small-cell lung cancer. Lancet 340: p. 145-146, 1992.

24. Nicolson, G.: Cancer Metastasis. Scientific Am 240: p. 66-76, 1979.

25. Perez CA, Devineni VR, Marcial-Vega V, Marks JE, Simpson JR, Kucik N.: Carcinoma of the nasopharynx: factors affecting prognosis. International Journal of Radiation Oncology, Biology, Physics. 23 (2): 271-280, 1992.

26. Polverini, P.J., Leibovich, S.J.: Induction of neovascularization in vivo and endothelial proliferation in vitro by tumor associated macrophages. Lab Invest 51: p. 635-642, 1984.

27. Rossi, A., Molinari, R., Boracchi, P. et al.: Adjuvant chemotherapy with vincristine, cyclophosphamide, and doxorubicin after radiotherapy in local-regional nasopharyngeal cancer: results of a 4-year multicenter randomized study. J Clin Oncol 6(9): p. 1401-10, 1988.

28. Sahin, A.A., Sneige, N., Ordonez, G.N.: Tumor angiogenesis detected by Ulex Europaeus agglutinin 1 lectin (UEA1) an factor VIII immunostaining in node-negative breast carcinoma (NNBC) treated by mastectomy: Prediction of tumor recurrence. Mod Pathol 6: p. 19a, 1993.

29. Schreiber, A.B., Winkler, M.E., Derynck, R.: Transforming growth factor-alpha: A more potent angiogenic mediator than epidermal growth factor. Science 232: p. 1250-1253, 1986.

30. Schuller, D.E., Metch, B., Stein, D.: Preoperative chemotherapy in advanced resectable head and neck cancer: Final report of the Southwest Oncology Group. Laryngo 98: p. 1205-1211, 1988.

31. Sneige, N., Singletary, E., Sahin, A.: Multiparameter analysis of potential prognostic factors in node negative breast cancer patients. Mod Pathol 5: p. 18a, 1992.

32. Srivastava, A., Laidler, P., Davies, R.: The prognostic significance of tumor vascularity in intermediate-thickness (0.76-4.0 mm thick) skin melanoma. Am J Pathol 133: p. 419-423, 1986.

33. Toi, M., Kashitani, J., Tominaga, T.: Tumor angiogenesis is a powerful prognostic indicator of primary breast carcinoma. Int J Cancer 55: p. 371-374, 1993.

34. Van Hoef, M., Knox, W.F., Dhesi, S.S.: Assessment of tumor vascularity as a prognostic factor in lymph node negative invasive breast cancer. Eur J Cancer 29A: p. 1141-1145, 1993.

35. Visscher, D.W., Smilanetz, S., Drozdowicz, S.: Prognostic significance of image morphometric microvessel enumeration in breast carcinoma. Analytical Quant Cytolol Histol 15: p. 88-92, 1993.

36. Wakui, S., Furusato, M., Itoh, T.: Tumor angiogenesis in prostatic carcinoma with and without bone marrow metastasis: A morphometric study. J Pathol 168: p. 257-262, 1992.

37. Weidner, N., Folkman, J., Pozza, F.: Tumor angiogenesis: A new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 84: p. 1875-1887, 1992.

38. Weidner, N., Semple, J.P., Welch, W.R.: Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 324: p. 1-8, 1991.

39. Weiss, L.: Biophysical aspects of the metastatic cascade. ed. Amsterdam: Holland, N., 1976.

40. Roychowdhury, D.F., Tseng, A., Fu, K.K., Weinverg V., and Weidner, N.: New prognostic factors in nasopharyngeal carcinoma. Proceedings of ASCO, 1995.

41. Fu K.K., Hammond E., Pajak T.F., et al. Flowcytometric quantification of proliferation associated nuclear antigen p105 and DNA content in advanced head & neck cancers: results of RTOG 91-08. Int J Radiat Oncol Phys 29: 661-671, 1994.

42. Kalbfleisch, J. D.; Prentice, R. L. The Statistical Analysis of Failure Time Data. New York: John Wiley and Sons; 1980.

43. Gray R.J.: A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 16: 1141-1154, 1988.

44. Kaplan E.L .,and Meier P. Nonparametric estimation from incomplete observations. J Amer Statist Assoc 53:457-481, 1958.

45. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chem Rep 5:163-170,1966.

46. Cox DR: Regression models and life tables. J Royal Stat Soc, Series B, vol 34:187-229, 1972.

* 47. Al-Sarraf, M, et al. Chemoradiotherapy vs Radiotherapy in Patients with Locally Advanced Nasopharyngeal Cancer. Phase III Randomized Intergroup Study (0099) (SWOG 8892, RTOG 8817, ECOG 2388). J Clin Oncol 16: 1310-1317, 1998.

* 48. Schoenfeld, D. (1981). The asymptotic properties of nonparametric tests for comparing survival distributions. *Biometrika*, 68, 316-319.

* 49. Perez, C.A and Brady, L.W., ed. Principles and Practice of Radiation Oncology, 3rd ed. Philadelphia: Lippincott-Raven; 1998.

*** Added 9/8/98**

APPENDIX I

RTOG 95-05

EVALUATION OF TUMOR,ANGIOGENESIS MEASURED WITH MICROVESSEL DENSITY *(MVD)* AS A PROGNOSTIC INDICATOR IN NASOPHARYNGEAL CARCINOMA

RESEARCH STUDY

I have the right to know about the procedures that are used in my participation in clinical research so I have an opportunity to decide whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm me; it is simply an effort to make me better informed so I may give or withhold my consent to participate in clinical research.

PURPOSE OF THE STUDY

Doctors will be looking at cells from my head and neck tumor to see if they can find special "markers" that can help them predict how a patient might respond to treatment for my type of cancer. An important part of this laboratory study is comparing the lab results with how I respond to treatment.

DESCRIPTION OF PROCEDURES

I have been invited to participate in this laboratory companion study. At the time of my radiation, some of my tumor was removed. As usual, part of the tumor went to the pathology department at the hospital for routine diagnosis. I am being asked for permission to use the remainder of the tumor and surrounding normal tissue for research purposes. Since the tissue was previously removed at the time of my treatment, the use of my tissue will not involve any additional procedures to me.

RISKS AND DISCOMFORTS

There is no additional risk to me if I agree to participate. The tumor and surrounding normal tissue were previously removed.

CONTACT PERSONS

I understand, however, I will not be provided with reimbursement for medical care other than what my insurance carrier has provided nor will I receive other compensation. For more information concerning the research, I can notify Dr. _____ the investigat
_____. In addition, I may contact _____
_____ at _____
for information regarding patients' rights in research studies.

BENEFITS

The results of this laboratory study will not directly affect me or my treatment plan. Doctors hope to use these results in the future to show which patients will best respond to therapy for head and neck cancer.

ALTERNATIVES

Participation in this study is entirely voluntary. I may choose not to participate. If I choose not to participate, my doctor will continue to give me the best care possible. I understand that I may ask questions and take as much time as needed to make my decision.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. No compensation for participation will be given. I understand that I am free to withdraw my consent to participate in this program at any time without prejudice to my subsequent care. Refusal to participate will involve no penalty, or loss of benefits. I am free to seek care from a physician of my choice at any time. If I do not take part in or withdraw from the study, I will continue to receive care if necessary.

CONFIDENTIALITY

I understand that records will be kept in a confidential form at this institution and also in a computer file at the headquarters of the Radiation Therapy Oncology Group *(RTOG)*. The confidentiality of the central computer record is carefully guarded. During their required reviews, representatives of the Food and Drug Administration *(FDA)*, the National Cancer Institute *(NCI)*, qualified representatives of applicable drug manufacturers, and other groups or organizations that have a role in the conduct of this study may have access to medical records which contain my identity. However, no information by which I can be identified will be released or published. Histopathologic material, including tissue and/or slides, will be sent to a central office for review and research investigation associated with this protocol.

I have read all of the above, asked questions, received answers concerning areas I did not understand, and willingly give my consent to participate in this program. Upon signing this form I will receive a copy.

_____ _____

Patient Signature *(or Legal Representative)* Date

APPENDIX II

APPENDIX IV

Institution # _____
RTOG 95-05 **ELIGIBILITY CHECK (9/8/98)**
Case # _____ ***(page 1 of 2)***

_____(Y) 1. Does the patient have biopsy-proven squamous cell carcinoma of the nasopharynx?
(Case will be made ineligible if submitted tissue is not from the nasopharynx)

_____(Y) 2. If not treated on an RTOG protocol, and treated with radiotherapy only, was the primary course of treatment given between 1975 and 1996?

_____(Y/NA) 3. If initial treatment included chemotherapy, was it administered according to Section 7.0.

4. Is the following information available?

_____(Y) a) Blocks and slides?

_____(Y) b) Tumor stage, treatment details, survival status?

_____(Y/NA) c) If treated with radiation only and currently alive, followup information including disease status?

_____(Y/NA) d) If dead at study entry, date of death and disease status?

_____(Y/NA) e) If treated with chemotherapy and currently alive, current disease status and available for yearly followup?

The following questions will be asked at Registration

_____(Y) 5. Was the Eligibility Checklist *(above)* completed?

_____(Y) 6. Is the patient eligible for this study?

_____(Y/N) 7. Has an RTOG 95-05 study-specific consent form been signed?

_____ If yes, date signed.

_____ If no, reason the consent was not signed?

1. patient not alive
2. patient signed consent form for treatment on prior RTOG study
3. other, specify _____

(continued on next page)

APPENDIX IV

Institution # _____
RTOG 95-05
Case # _____

ELIGIBILITY CHECK (9/8/98)
(page 2 of 2)

_____ 8. On which previous RTOG protocol was the patient treated? *(79-13, 79-15, 83-13, 85-27 or 88-17 [INT 0099])*

_____ 9. Previous RTOG case number?

_____ Patient's Name

_____ Verifying Physician

_____ Patient ID #

_____ Referring Institution # *(if different)*

_____ Prior Treatment or Study Group

1. Prior RTOG Study-RT Only
2. Not an RTOG Study-RT Only
3. On 88-17 or Non-RTOG Study plus Chemo

_____ Birthdate

_____ Sex

_____ Race

_____ Social Security Number

_____ Zip Code *(9 digit if available)*

_____ Method of Payment *(original treatment)*

_____ Did the patient get any treatment at an VA or military facility?

_____ Registration Date

Completed by _____ Date _____