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# Phase 1 Trial of Bevacizumab With Concurrent Chemoradiation Therapy for Squamous Cell Carcinoma of the Head and Neck With Exploratory Functional Imaging of Tumor Hypoxia, Proliferation, and Perfusion

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# Abstract

**Purpose**—A phase 1 trial was completed to examine the safety and feasibility of combining bevacizumab with radiation and cisplatin in patients with locoregionally advanced squamous cell carcinoma of the head and neck (HNSCC) treated with curative intent. Additionally, we assessed

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the capacity of bevacizumab to induce an early tumor response as measured by a series of biological imaging studies.

**Methods and Materials**—All patients received a single induction dose of bevacizumab (15 mg/kg) delivered 3 weeks (±3 days) before the initiation of chemoradiation therapy. After the initial dose of bevacizumab, comprehensive head and neck chemoradiation therapy was delivered with curative intent to 70 Gy in 33 fractions with concurrent weekly cisplatin at 30 mg/m<sup>2</sup> and bevacizumab every 3 weeks (weeks 1, 4, 7) with dose escalation from 5 to 10 to 15 mg/kg. All patients underwent experimental imaging with [<sup>18</sup>F]fluorothymidine positron emission tomography (FLT-PET) (proliferation), [<sup>61</sup>Cu]Cu-diacetyl-bis(N4-methylthiosemicarbazone) PET (Cu-ATSM-PET) (hypoxia), and dynamic contrast-enhanced computed tomography (DCE-CT) (perfusion) at 3 time points: before bevacizumab monotherapy, after bevacizumab monotherapy, and during the combined therapy course.

**Results**—Ten patients were enrolled. All had stage IV HNSCC, all achieved a complete response to treatment, and 9 of 10 remain alive, with a mean survival time of 61.3 months. All patients experienced grade 3 toxicity, but no dose-limiting toxicities or significant bleeding episodes were observed. Significant reductions were noted in tumor proliferation (FLT-PET), tumor hypoxia (Cu-ATSM-PET), and DCE-CT contrast enhancement after bevacizumab monotherapy, with further decreases in FLT-PET and Cu-ATSM-PET during the combined therapy course.

**Conclusions**—The incorporation of bevacizumab into comprehensive chemoradiation therapy regimens for patients with HNSCC appears safe and feasible. Experimental imaging demonstrates measureable changes in tumor proliferation, hypoxia, and perfusion after bevacizumab monotherapy and during chemoradiation therapy. These findings suggest opportunities to preview the clinical outcomes for individual patients and thereby design personalized therapy approaches in future trials.

# Introduction

Head and neck squamous cell carcinoma (HNSCC) represents a heterogeneous group of tumors that involve the oral cavity, oropharynx, nasopharynx, hypopharynx, larynx, and paranasal sinuses. Despite stepwise advances in the treatment of HNSCC, the outcomes remain modest for patients with advanced-stage disease, with 5-year absolute survival rates on the order of 30% to 50% (1). Recently, targeted therapies directed against the epidermal growth factor receptor, integrated with radiation therapy (RT) or chemotherapy, have shown effectiveness in improving overall survival in the definitive (1) and metastatic/recurrent setting (2). These results provide evidence that a molecularly targeted therapy can enhance the efficacy of curative RT and cytotoxic chemotherapy in the metastatic/recurrent setting.

Rich vascular supply and overexpression of vascular endothelial growth factor (VEGF) receptors are common in HNSCC and are indicative of a poor prognosis (3). Tumor hypoxia is prevalent in HNSCC (4) and is associated with poor outcome after radiation (5). Antiangiogenic therapy has been postulated to improve hypoxia status and thereby improve patient outcome in this setting (6). Bevacizumab is an anti-VEGF monoclonal antibody that has been approved by the U.S. Food and Drug Administration in several solid tumor

settings. Recent preclinical studies have demonstrated a synergistic effect between RT and bevacizumab for reducing proliferation in HNSCC tumor models (7, 8).

To date, limited studies examining bevacizumab in combination with cisplatin-based chemoradiation therapy in HNSCC have been performed. We completed a phase 1 dose escalation trial with the primary objective of examining the safety and feasibility of combining bevacizumab with RT and cisplatin in patients with locoregionally advanced HNSCC. The secondary objectives included time to disease progression and survival and ability of bevacizumab to affect biological imaging surrogates of tumor hypoxia, proliferative capacity, and tumor perfusion. We report the clinical outcomes and the correlative imaging results.

#### Methods and Materials

#### Patients

Patients with confirmed diagnoses of locoregionally advanced SCC of the oropharynx, hypopharynx, or larynx (stage III/IV disease) were prospectively enrolled in a phase 1 trial. The eligibility criteria are described in Supplement E1 (available online at www.redjournal.org). The trial was approved by the University of Wisconsin Scientific Review Committee and Institutional Review Board. All patients provided study-specific informed consent.

#### **Chemotherapy delivery**

All patients received a single induction dose of bevacizumab (15 mg/kg) delivered 3 weeks ( $\pm$ 3 days) before the initiation of chemoradiation therapy (Fig. 1). Three weeks ( $\pm$ 3 days) after receiving induction bevacizumab, patients began combined therapy consisting of comprehensive RT, 7 weekly doses of cisplatin at 30 mg/m<sup>2</sup>, and 3 doses of bevacizumab every 3 weeks as follows: 5 mg/kg for the first 3 patients, 10 mg/kg for the next 2 patients, and 15 mg/kg for the final 5 patients as tolerated. Patients were not enrolled in the 10 mg/kg or 15 mg/kg cohorts until 3 weeks after the previous cohort had been completed. This escalation scheme represented a modest acceleration of dose relative to the conventional 3 + 3 schedule, and it allowed limiting the number of patients on protocol to 10. Cisplatin was administered with antiemetics, hydration, and electrolyte support according to standard institutional practice. Dose-limiting toxicity (DLT) was defined as any grade 4 adverse event at least possibly related to bevacizumab within 90 days of protocol treatment according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0.

#### Radiation treatment planning and delivery

All patients underwent a treatment planning computed tomography (CT) scan with intravenous contrast medium. Customized thermoplastic masks were made to immobilize each patient's head and neck. Primary areas of gross disease and positive lymph nodes were planned as gross tumor volume, receiving 70 Gy in 33 fractions. The high-risk planning target volume (PTV) encompassed the gross tumor volume plus a margin and the regional lymphatics at highest risk for subclinical disease, receiving 60 to 64 Gy in 33 fractions. In

patients with unilateral nodal disease, a lower-risk PTV encompassed the contralateral-neck lymphatics, receiving 54 Gy in 33 fractions. An additional lower-risk PTV encompassed the lower neck and supraclavicular lymphatics, receiving 50 Gy in 33 fractions. Normal anatomic structures, including the parotid glands, spinal cord, brainstem, optic nerves, mandible, larynx, oral cavity, and lens, were contoured for dose limitation. All patients were treated with intensity modulated radiation therapy with cone-beam or megavoltage CT image guidance before each treatment.

#### Surgery

Diagnosis was established by biopsy of the primary tumor or fine needle aspiration of metastatic lymph nodes before enrollment. No definitive surgery was performed in any patient. Patients were eligible for neck dissection 8 to 12 weeks after the completion of therapy.

#### **Imaging studies**

All patients underwent experimental imaging for proliferation, hypoxia, and perfusion before bevacizumab monotherapy and 3 weeks after bevacizumab induction, with proliferation and hypoxia imaging repeated 1 to 2 weeks into the combined therapy course (Fig. 1).

Proliferation imaging was performed with [<sup>18</sup>F]fluorothymidine (FLT) positron emission tomography (PET)/CT (9), which has shown utility for measuring RT response in HNSCC (10). Hypoxia imaging was performed with [<sup>61</sup>Cu] Cu-diacetyl-bis(N4methylthiosemicarbazone) (Cu-ATSM-PET/CT) (11), which has shown a predictive relationship to response and survival in a variety of settings, including HNSCC (12). Perfusion imaging was performed with dynamic contrast-enhanced CT (DCE-CT), which measures blood volume, blood flow, and microvascular permeability through cine imaging of the passage of injected contrast medium through the vascular system and into tumor areas. DCE-CT has been shown to quantify changes after RT in HNSCC (13) and also after bevacizumab in rectal cancer (14). The imaging protocols are described in Supplement E1 (available online at www.redjournal.org).

Paired *t* tests were performed to test for statistical significance between imaging metrics ( $\alpha = 0.05$ ). The PET image metrics included SUV<sub>mean</sub>, the average uptake in the tumor region; SUV<sub>peak</sub>, the average uptake in a 1.0 cm<sup>3</sup> sphere that encompassed the region of greatest uptake (15); and <sub>K</sub>FLT (net FLT flux), the rate parameter that describes phosphorylation of FLT in the cell after the efflux of tracer from plasma into tissue (16). Additionally, the PERCIST (PET Response Criteria In Solid Tumors) guidelines (15) were adapted in such a manner that patients who showed a greater than 30% decrease in uptake were classified as responders. The tested DCE-CT image metrics included CT enhancement, mean transit time, blood volume, blood flow, and permeability-surface-area-product.

# Results

#### Patients

Between 2007 and 2010, 10 patients with locoregionally advanced HNSCC were enrolled (Table 1). All patients had stage IV HNSCC. Although testing for human papillomavirus (HPV) was not incorporated into trial eligibility, retrospective tissue analyses demonstrated that tumors from all patients stained positively for p16, a surrogate for HPV positivity. Although patients with tumors of the oropharynx, hypopharynx, or larynx were eligible for enrollment, all 10 patients accrued had cancer of the oropharynx. This limitation of enrollment to the oropharynx was unintentional but was influenced by the high prevalence of patients with cancer of the oropharynx receiving primary radiation or chemoradiation at our institution.

#### Treatment

Thirty-three infusions of bevacizumab were administered. One patient received 1 of 4 infusions, 4 patients received 3 of 4, and 5 patients received 4 of the 4 planned infusions. Dose reductions of bevacizumab were not permitted. Sixty infusions of weekly cisplatin were administered, for an average of 6 of the 7 planned infusions per patient, with a mean dose intensity of 25.6 mg/m<sup>2</sup>/week.

#### Dose-limiting toxicities and maximum tolerated dose

The conditions of all patients were evaluable. No DLTs were observed in any patient, such that the maximum tolerated dose (MTD) identified was 15 mg/kg.

#### Dose escalation, toxicity, and safety

The conditions of all patients were evaluable. No deaths within 30 days or during the study were noted. Grades 2, 3, and 4 toxicities at least possibly related to bevacizumab are shown in Table 2.

Serious adverse events occurred in 2 patients. A 59-year-old man receiving bevacizumab at 5 mg/kg was hospitalized after his third dose of bevacizumab and fourth dose of weekly cisplatin because of refractory grade 3 nausea, vomiting, and dehydration despite maximal outpatient support, including daily intravenous fluids. He received a diagnosis of candidal esophagitis and required parenteral nutrition. He subsequently completed 2 additional doses of weekly cisplatin at 20 mg/m<sup>2</sup> and his final dose of bevacizumab. A second patient, a 75year-old man receiving bevacizumab at 15 mg/kg, was hospitalized because of grade 1 oral cavity hemorrhage, grade 1 thrombocytopenia, and grade 1 anemia. His platelet count and hemoglobin were within normal limits at study entry, but both fell to grade 1 after his third dose of bevacizumab and fourth dose of weekly cisplatin. He had no history of bleeding diathesis, and coagulation assays were unremarkable at enrollment and admission. Endoscopic evaluation detected only a blood clot at the base of the tongue, with no active bleeding, and vascular imaging demonstrated findings consistent with tumor necrosis but no frank bleeding. There was no evidence of lower gastrointestinal bleeding, he did not require blood product support, and his bleeding did not recur. The patient declined further bevacizumab and cisplatin, and he completed his radiation without further hemorrhage.

Subsequent doses of bevacizumab were not given to a patient after the induction dose at 5 mg/kg because of persistent grade 3 hypertension despite maximal support. Four patients missed the fourth dose of bevacizumab because of the following: asymptomatic grade 3 hyponatremia in a patient with a lengthy history of hypopituitarism (10 mg/kg dose level); asymptomatic grade 3 neutropenia (15 mg/kg); the previously described oral cavity hemorrhage (15 mg/kg); and an acute lower-extremity deep vein thrombosis in a patient without a history of thromboembolic events (15 mg/kg). Two patients experienced grade 3 tumor pain almost immediately after receiving the initial dose of induction bevacizumab, 1 each at 5 mg/kg and 10 mg/kg. This pain persisted in the patient receiving 5 mg/kg throughout the successful completion of all therapies, ultimately requiring doses of topical fentanyl at 300 µg/h (the patient was previously opioid naïve). Grade 2 hypertension was successfully treated in 3 patients. No cases of fistula formation or arterial thromboses were detected.

Cisplatin was dose-reduced or omitted because of the previously described admission for grade 3 nausea, vomiting, and dehydration (5 mg/kg dose level), asymptomatic grade 3 hyponatremia (10 mg/kg), asymptomatic grade 3 neutropenia (15 mg/kg), the previously described oral cavity hemorrhage (15 mg/kg), and asymptomatic grade 1 thrombocytopenia in the patient with the newly diagnosed deep vein thrombosis of the lower extremity (15 mg/kg). This patient subsequently received anticoagulant therapy, which was successful and without adverse events. There were no cases of febrile neutropenia during the study or any findings of grade 2 or greater renal dysfunction, tinnitus, or peripheral sensory neuropathy.

The only patient who declined subsequent systemic anticancer treatment because of experiencing an adverse event was the patient with the oral cavity hemorrhage. The fourth dose of bevacizumab and the final 2 doses of cisplatin were discontinued at the discretion of the treating physician in the patient with the asymptomatic grade 3 hyponatremia.

#### **Radiation toxicity**

All patients successfully completed radiation treatment at the prescribed doses. We observed grade 2 to grade 3 acute mucositis and dysphagia in all 10 patients in the study, possibly exacerbated by bevacizumab. Mucositis resolved in all but 1 patient by 3 months after treatment. This patient (receiving 5 mg/kg bevacizumab) had eventual resolution of his prolonged mucositis at 9 months. As described previously, he experienced severe tumor bed pain (base-of-tongue region) from the time of his induction bevacizumab, ultimately requiring high-dose fentanyl. His tumor site underwent biopsy and gentle debridement at 3 and 6 months, and the results of pathologic analysis remained negative. This site healed completely by 11 months with full resolution of his pain. No patient retained a feeding tube beyond 12 months after treatment completion; 2 patients required soft mechanical diet for up to 14 and 18 months, respectively, and then resumed solid food intake. One patient had a small mandibular bone exposure at 21 months that resolved completely after in-office debridement of a small bony spicule and 4 weeks of oral antibiotic therapy. No severe late adverse events of bone or soft tissue were observed in the 10 patients after a mean follow-up time of 61.3 months.

#### **Oncologic outcomes**

At the last follow-up visit, 9 of 10 patients were alive. Only 1 patient underwent post chemoradiation neck dissection, with no evidence of residual carcinoma. Durable local control after the primary treatment regimen was obtained in 9 of 10 patients; 1 patient with a T3N2b tongue base tumor experienced local recurrence at 15 months, received salvage composite resection, and is now without evidence of disease 50 months later. Two patients experienced lung metastases at 16 and 18 months, respectively, 1 of whom expired at 68 months. The median progression-free survival for the 10 patients was 50.1 months.

#### Imaging results

Twenty-eight PET-CT and 20 DCE-CT scans were obtained of the 10 patients; 1 patient did not receive pretreatment FLT-PET and another did not receive pretreatment Cu-ATSM-PET (Fig. 1). Images from a representative patient are highlighted in Figure 2. Significant changes were observed over all patients in terms of proliferation, hypoxia, and CT enhancement (Fig. 3).

**FLT-PET (proliferation)**—Significant decreases after bevacizumab monotherapy were measured in SUV<sub>mean</sub> ( $2.2 \pm 0.8$  to  $1.5 \pm 0.2$ , P = .008), SUV<sub>peak</sub> ( $7.5 \pm 2.3$  to  $5.1 \pm 1.7$ , P = .05), and K<sub>FLT</sub> ( $0.04 \pm 0.01$  to  $0.03 \pm 0.01$ , P < .001). Eight of 9, 6 of 9, and 5 of 9 patients were classified as responders to bevacizumab monotherapy at the 30% level when evaluated with K<sub>FLT</sub>, SUV<sub>mean</sub>, and SUV<sub>peak</sub>, respectively. After 1 to 2 weeks of combined therapy, FLT uptake demonstrated further significant decline in SUV<sub>mean</sub> ( $1.5 \pm 0.2$  to  $0.9 \pm 0.4$ , P < .001, 8/10 response) and SUV<sub>peak</sub> ( $5.0 \pm 1.6$  to  $2.8 \pm 2.0$ , P < .001, 8/10 response). However, K<sub>FLT</sub> did not demonstrate a significant reduction after the beginning of combined therapy (P = .90, 3/9 response).

**Cu-ATSM-PET (hypoxia)**—The decrease in SUV<sub>mean</sub> after bevacizumab monotherapy was significant, from  $1.5 \pm 0.4$  to  $1.3 \pm 0.2$  (P = .046); the group means of SUV<sub>peak</sub> decreased from  $3.4 \pm 1.4$  to  $3.0 \pm 0.9$ , but the change was not significant (P = .24). Two of 9 patients demonstrated a greater than 30% response in SUV<sub>mean</sub> or SUV<sub>peak</sub>. After 1 to 2 weeks of combined therapy, SUV<sub>mean</sub> decreased significantly from  $1.3 \pm 0.2$  to  $1.1 \pm 0.2$  (P = .048, 4/10 response). Again, SUV<sub>peak</sub> uptake decreased, but the changes were not significant (P = .11, 3/10 response).

**DCE-CT (vascularity, perfusion, permeability)**—After bevacizumab monotherapy, a significant decrease in CT enhancement was observed (96 ± 6 to 77 ± 3 Houndsfield units; P = .02). Blood flow (100 ± 10 to 83 ± 8 mL/100 g/min; P = .09) and blood volume (6.2 ± 0.5 to 5.5 ± 0.6 mL/100 g; P = .10) showed trends toward significant changes. No significant trends were observed for mean transit time (P = .93) or permeability-surface-area-product (P = .95).

## Discussion

Antiangiogenic therapies hold promise for many solid tumors. To date, the data are limited regarding the use of bevacizumab in conjunction with chemoradiation therapy for locally

advanced HNSCC treated with curative intent. We demonstrate that the incorporation of bevacizumab with comprehensive cisplatin-based chemoradiation therapy for HNSCC appears safe and feasible. Additionally, novel correlative imaging results indicate that bevacizumab can affect HNSCC tumors by reducing tumor proliferative capacity, reducing hypoxia, and having an impact on blood flow and volume, providing evidence for biological mechanisms by which bevacizumab may augment the efficacy of chemoradiation therapy in HNSCC.

Initial studies investigating bevacizumab in conjunction with RT and concurrent fluorouracil and hydroxyurea chemotherapy in a phase 1 study with a large proportion of patients who had previously received RT reported problematic late tissue adverse events, including fistula formation and tissue ulceration/necrosis (17). A follow-up phase 2 study performed in treatment-naïve patients was terminated early because of unexpected failure in the bevacizumab arm, with 2-year survival rates of 89% in the patients treated with twice-daily RT, hydroxyurea, and fluorouracil compared with 58% in patients treated with the same regimen plus concurrent bevacizumab (18). Recently, more favorable reports of safety and efficacy have been published for bevacizumab, cisplatin, and radiation (19, 20) and for bevacizumab, erlotinib, and radiation (21, 22), with 2-year locoregional control and overall survival on the order of 80% and 90%. These important trials suggest good feasibility and promise for bevacizumab in advanced HNSCC, and a potential delay in subclinical distant disease for patients with cancer of the nasopharynx.

The current study, using dose-escalated bevacizumab in conjunction with weekly cisplatin and modestly accelerated daily radiation (2.12 Gy per fraction), demonstrated acceptable safety and tolerability, including at the MTD of 15 mg/kg bevacizumab, with all patients successfully completing RT and no occurrences of DLT. Our dose range and frequency of administration of bevacizumab were based on contemporaneous concurrent bevacizumabchemotherapy-radiation therapy trials in the curative setting for various solid tumors, with treatment doses ranging from 2.5 to 10 mg/kg every 14 or 21 days (17, 23, 24).

The addition of bevacizumab likely modestly exacerbated toxicities in our population, although comparisons are limited by our sample size and the fact that reporting on this weekly schedule of cisplatin derives primarily from single-institution retrospective analyses (25–28). Grade 2 to grade 3 mucositis and dysphagia were detected in all patients, as is typical for HNSCC patients receiving 70 Gy radiation with concurrent cisplatin. Worsening of clinically significant oral mucositis was not increased in the study by Salama et al (18) or in a preclinical mouse model in which anti-VEGF antibody was added to oral irradiation (29). However, given the propensity of antiangiogenic therapies to cause ischemic mucosal injury throughout the gastrointestinal tract, close monitoring of mucosal integrity and function is warranted in future clinical trials.

We observed fewer hematologic toxicities in our population than were noted in retrospective series of weekly cisplatin without bevacizumab, with no cases of febrile neutropenia or thrombocytopenia exceeding grade 1. Similarly, the rates of nausea, emesis, dehydration, and fatigue were not markedly different from those in retrospective reports of weekly cisplatin use. The dose intensity of cisplatin was similar to that achieved at other centers,

despite our addition of bevacizumab (25–28). Improved or at least similar tolerability of our triple-modality therapy may have been unintentionally biased as a result of the uniform enrollment of patients whose tumors were positive for p16, suggesting a more robust baseline health status (30). However, in as much as the addition of bevacizumab to concurrent chemoradiation therapy for HNSCC has been shown to exacerbate hematologic events when cisplatin is administered every 21 days (19), incorporating it in chemotherapy regimens with lower weekly doses of cisplatin may be more tolerable.

Two patients experienced grade 3 tumor pain almost immediately upon receiving the singleagent induction dose of bevacizumab, requiring high-dose opioids throughout treatment for 1 patient. Increased pain attributed at least possibly to bevacizumab has been described in multiple malignancies, usually manifesting as bowel or organ ischemia, perforation, or both (31, 32). Bevacizumab is associated with both hemorrhagic and thrombotic adverse events, possibly related to endothelial cell damage from inhibition of VEGF, which is necessary for maintenance of functional endothelium. Endothelial disruption leads to exposure of the prothrombotic basement membrane and bleeding from the loss of integrity of the endothelium (33). We speculate that these disruptive effects on endothelium adjacent to the malignant and supportive cells may have contributed to the pain these patients experienced after bevacizumab infusion.

Locoregional control in our population was 90% after primary therapy, and median progression-free survival exceeded 50 months. These efficacy outcomes are favorable and similar to other single-institution series describing the use of chemoradiation therapy with weekly cisplatin, including our previously published series (25). The fact that all patients were ultimately confirmed to have p16-positive tumors, strongly suggesting HPV positivity, also likely contributed to the excellent overall outcome observed in this pilot study. Additionally, it is noted that the rate of study accrual was relatively slow because the large number of additional imaging sessions required highly motivated patients, possibly representing a source of participation bias.

The majority of patients showed strong reduction in tumor proliferation after bevacizumab monotherapy as assessed by FLT-PET (8/9, 6/9, and 5/9 responders at the 30% level for  $K_{FLT}$ , SUV<sub>mean</sub>, and SUV<sub>peak</sub>, respectively). After 1 to 2 weeks of chemoradiation therapy, all patients had demonstrated a significant proliferative response as measured by mean FLT-PET uptake. These results mirror recent preclinical studies demonstrating synergy between bevacizumab and RT in reducing proliferation in head and neck models (7, 8, 34, 35). Accelerated repopulation is a major contributor to radioresistance in HNSCC (36). These observations suggest a mechanism by which bevacizumab may affect HNSCC radiosensitivity. Therefore, further investigation into the utility of FLT-PET imaging as an early predictor of favorable response to antiangiogenic therapy in a larger study may be warranted.

The reduction of intratumoral hypoxia after bevacizumab monotherapy appears to be driven by strong responses in a subset of patients, whereas other patients demonstrated modest decline or increase in Cu-ATSM-PET. This may correspond with a disparity in the preclinical literature, where some evidence suggests that normalization of pathologic blood

vessels can enhance oxygen delivery and reduce tumor hypoxia (37, 38), whereas other data suggest that enhanced tumor responses result from reduced blood vessel penetration and nutrient delivery, with enhanced rates of tumor hypoxia and resultant necrosis (34, 39). A contributing factor may be that the distribution of hypoxic phenotype has been shown to be variable in HNSCC patients (40). Further studies are needed to better define the impact of bevacizumab on hypoxia, and ultimately tumor control, in HNSCC.

Reports of imaging assessment of perfusion and vascularity in HNSCC patients after bevacizumab are limited. The DCE-CT results in the current study coincide with the DCE-MRI results of Yoo et al (21), who showed changes in Ktrans (perfusion) after bevacizumab, erlotinib, and RT. DCE-CT perfusion rate was previously reported to predict outcomes for HNSCC after RT only (13). We add to these reports our findings of decreased CT enhancement after bevacizumab, with trends toward decreased regional blood flow and blood volume.

In conclusion, the incorporation of bevacizumab into a regimen of curative-intent chemoradiation therapy for HNSCC appears safe and feasible, although with some increase in the acute toxicity profile associated with concurrent weekly cisplatin. Experimental imaging demonstrates clear changes in tumor proliferation, hypoxia, and perfusion in HNSCC tumors after bevacizumab alone and during combined therapy. These findings highlight the potential of novel imaging modalities to identify early tumor response in selected HNSCC patients treated with antiangiogenic therapies. In the future, this approach may provide opportunities to personalize curative treatment regimens based on the early tumor response profile for individual patients.

# **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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#### Summary

This phase 1 study reports toxicity data and clinical outcome data regarding the integration of bevacizumab into curative chemoradiation regimens for squamous cell cancer of the head and neck (HNSCC). Additionally, biological imaging studies designed to assess the effects of bevacizumab on tumor proliferation, hypoxia, and perfusion are reported, which may help to identify mechanisms by which antiangiogenic therapies act in HNSCC patients. These findings suggest opportunities to preview the clinical outcomes and to personalize therapy for individual patients in future trials.



### Fig. 1.

Design of phase 1 study incorporating dose-escalated bevacizumab and biological imaging into chemoradiation therapy course for locally advanced squamous cell carcinoma of the head and neck. BEV =bevacizumab; CDDP =  $30 \text{ mg/m}^2$  cisplatin; CU-ATSM-PET = [ $^{61}$ Cu] Cu-diacetyl-bis(N4-methylthiosemicarbazone) positron emission tomography; DCE-CT = dynamic contrast-enhanced computed tomography; FLT-PET = [ $^{18}$ F]fluorothymidine positron emission tomography.

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#### Fig. 2.

Representative images of [<sup>61</sup>Cu]Cu-diacetyl-bis(N4-methylthiosemicarbazone) positron emission tomography (Cu-ATSM-PET), [<sup>18</sup>F]fluorothymidine positron emission tomography (FLT-PET), and dynamic contrast-enhanced computed tomography (DCE-CT) in patient 8 before bevacizumab monotherapy (time point 1), after 3 weeks of bevacizumab (time point 2), and after 1 to 2 weeks of chemoradiation therapy (time point 3). Measureable reductions in hypoxia, proliferation, and CT enhancement were observed in response to bevacizumab monotherapy and combined therapy. This patient achieved a complete

response as shown by FLT-PET and Cu-ATSM-PET after bevacizumab monotherapy and remains disease free at 53 months.



#### Fig. 3.

Changes in [<sup>61</sup>Cu]Cu-diacetyl-bis(N4-methylthiosemicarbazone) positron emission tomography (Cu-ATSM-PET), [<sup>18</sup>F]fluorothymidine positron emission tomography (FLT-PET), and dynamic contrast-enhanced computed tomography (DCE-CT) in response to bevacizumab monotherapy and chemoradiation therapy before bevacizumab monotherapy (time point 1), after 3 weeks of bevacizumab (time point 2), and after 1 to 2 weeks of chemoradiation therapy (time point 3).

#### Table 1

#### Patient and tumor characteristics

Characteristic	n
Sex	
Male	9
Female	1
Age, y	
Median (range)	60 (43–75)
Stage	
T2 N2b	1
T2 N2c	3
T3 N2b	2
T4 N2b	2
T4 N2c	2
Location	
Base of tongue	6
Tonsil	4
HPV status (p16)	
Positive	10
Negative	0
Tobacco (>10 pack years)	
Yes	6
No	4
Active tobacco use	
Yes	2
No	8

Abbreviation: HPV = human papillomavirus.

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# Table 2

Number of patients experiencing acute adverse events at least possibly related to bevazicumab in combination with concurrent cisplatin and radiation therapy

			Be	vaci	zumz	ab de	ose		
	ν <sup>–</sup>	mg/l n = 3	8 8	10	n = 2	) kg	15	n = 5	kg (
	l		I I		Grad	e			1
Adverse event	7	e	4	7	e	4	7	Э	4
Hematologic									
Leukopenia	1			Ч			i.	-	1
Neutropenia	'	,	,			ī	ī	-	,
Nonhematologic									
Mucositis	1	З	,		7	i.	7	З	1
Dysphagia	'	$\mathfrak{S}$	,	·	0	,	0	З	,
Tumor pain	,	-		-	-		З		1
Hypertension	1	-	,	7		i.	7	,	1
Cellulitis	-	,	,			ī	ī	,	,
Weight loss	1	7			-		б		
Fatigue	7			-		,	-		1
Dehydration	'	-		0			ı		,
Nausea	1	-			-	,	-		
Emesis	'	1		-			З		1
Oral pain	'								,
Skin reaction	'	,	,	,	-	,	,	,	1
Gastritis	1	,	,	-		i.	i.	,	1
Dysgeusia	'					ŀ	0		,
Hyponatremia	'					,	,		
Candidiasis	7					,	,		1
Diarrhea	'					ŀ	-		,
Anorexia	ľ	-	,	,		,	,	,	

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 $\begin{array}{l} 15 mg/kg \\ (n=5) \end{array}$ 

	ري 1	mg/k	ສ <sub>ອ</sub> ຼ		mg/]		12
					rade	、 <b> </b> 。	Í I
Adverse event	7	e	4	6	e	4	6
Thrombosis							
Hemorrhage, oral cavity							-
Leg edema					1		

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