Phase II Trial of Pemetrexed and Bevacizumab in Patients With Recurrent or Metastatic Head and Neck Cancer

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ABSTRACT

Purpose

We hypothesized that bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF), will potentiate the activity of pemetrexed, a multitargeted antifolate, in squamous cell carcinoma of the head and neck (SCCHN).

Patients and Methods

Patients with previously untreated, recurrent, or metastatic SCCHN were treated with pemetrexed 500 mg/m² and bevacizumab 15 mg/kg given intravenously every 21 days with folic acid and B₁₂ supplementation until disease progression. Primary end point was time-to-progression (TTP). DNA was isolated from whole blood samples for the detection of polymorphisms in thymidylate synthase, methylenetetrahydrofolate reductase (*MTHFR*), and *VEGF*.

Results

Forty patients were enrolled. The median TTP was 5 months, and the median overall survival (OS) was 11.3 months. In 37 evaluable patients, the overall response rate was 30%, including a complete response rate of 5%, and the disease control rate was 86%. Grade 3 to 5 bleeding events occurred in six patients (15%): four were grade 3, and two were fatal. Other serious toxicities in 10% or more of patients included neutropenia (10%) and infection (12.5%). One patient died of sepsis after receiving eight cycles of therapy. For the *MTHFR* A1298C (rs1801131) single nucleotide polymorphisms, homozygote patients with AA had worse OS (*P* = .034).

Conclusion

The addition of bevacizumab to pemetrexed resulted in promising efficacy outcomes in SCCHN. Bleeding events were frequent but some may have been due to natural history of disease. Polymorphisms in *MTHFR* may offer potential for treatment individualization.

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INTRODUCTION

Approximately 47,000 new cases of head and neck cancer are diagnosed annually in the United States, most of which are histologically squamous cell carcinomas.¹ Squamous cell carcinoma of the head and neck (SCCHN) is potentially curable when diagnosed at early or localized stages. Distant metastases, which commonly involve the lungs, are seen in a small fraction of patients at first presentation but may subsequently develop in approximately 20% to 30% of patients who initially present with locally advanced SCCHN. Patients with recurrent or metastatic SCCHN have a poor prognosis with a median survival of 6 to 10 months.^{2,3} Selected patients with locally recurrent disease can be treated with a curative intent with locoregional therapies, such as salvage surgery or radiotherapy; however, the vast majority die of their disease.^{2,3}

Active single agents in SCCHN include methotrexate, bleomycin, cisplatin, carboplatin, FU, paclitaxel, docetaxel, and cetuximab.⁴ A small randomized study⁵ reported survival benefit for chemotherapy with cisplatin versus no treatment. Although combination chemotherapy yields higher response rates, it has not been shown to produce a survival benefit compared with single agents in randomized comparisons.⁶⁻⁸ Moreover, toxicity was increased with combination chemotherapy, especially with cisplatin-based regimens. Recently, the addition of cetuximab to platinum and FU was shown to improve median survival from 7.4 to 10.1 months and median progression-free survival from 3.3 to 5.6 months in patients with recurrent or metastatic SCCHN, albeit with increased but acceptable toxicities.⁹ The study of other novel agents remains of major importance for the treatment of recurrent or metastatic SCCHN.

Pemetrexed is a multitargeted antifolate that inhibits several enzymes of the folate pathway including thymidylate synthase (TS), dihydrofolate reductase, and glycylamide ribonucleotide formyl transferase.¹⁰ It has proven efficacy in non–small-cell lung cancer^{11,12} and malignant pleural mesothelioma.¹³ Because methotrexate, another antifolate, is a standard therapy for recurrent or metastatic SCCHN, the development of pemetrexed for the treatment of SCCHN has attracted the attention of clinical investigators. A phase II trial of pemetrexed 500 mg/m² every 3 weeks reported an objective response rate of 27% and a median time-to-progression (TTP) of 3.9 months in patients with recurrent or metastatic SCCHN.¹⁴ A recently presented phase III trial showed that the combination of pemetrexed and cisplatin does not significantly prolong survival over cisplatin alone in recurrent or metastatic SCCHN; however, survival benefit was detected in the subset of patients with good performance status or oropharyngeal primaries.¹⁵

Angiogenesis is critical for tumor growth, and vascular endothelial growth factor (VEGF) is the most important proangiogenic factor.^{16–18} Targeting angiogenesis by using bevacizumab, a monoclonal antibody against VEGF, has been efficacious in several solid tumors. There is strong evidence for increased antitumor efficacy when bevacizumab is added to various chemotherapeutics, and survival benefit with this approach has been demonstrated in metastatic colorectal cancer and non–small-cell lung cancer.¹⁹ One possible mechanism of action is by increasing delivery of chemotherapy to the tumor site.^{20,21} VEGF and other angiogenesis markers are expressed in SCCHN, and high VEGF levels have been correlated with poor survival.^{22–24}

Gene polymorphisms of TS and methylenetetrahydrofolate reductase (*MTHFR*), a key enzyme in folate metabolism that regulates nucleic acid methylation, have been correlated with outcome in patients treated with antifolates; however, results have been inconsistent.²⁵ The clinical relevance of these polymorphisms in patients with SCCHN treated with pemetrexed remains uncertain. Additionally, there are no reliable biomarkers that can be used as predictors of outcome after antiangiogenesis treatment. An association between VEGF genotype and survival was noted in a trial of paclitaxel and bevacizumab in patients with breast cancer.²⁶ In this phase II study, we investigated the hypothesis that bevacizumab can potentiate the activity of pemetrexed in patients with recurrent or metastatic SCCHN. We also evaluated TS, *MTHFR*, and VEGF gene polymorphisms and their association with toxicity and efficacy.

PATIENTS AND METHODS

Patient Selection

Eligible patients were age 18 years or older with metastatic or locally recurrent SCCHN, Eastern Cooperative Oncology Group (ECOG) performance status 0 to 1, and measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) definitions.²⁷ No prior chemotherapy or biologic therapy for recurrent or metastatic SCCHN and no prior pemetrexed or bevacizumab at any time were allowed. Prior chemotherapy and targeted agents (eg, cetuximab) as part of initial potentially curative therapy was permitted provided it was completed > 6 months before registration. Patients were also required to have absolute neutrophil count > 1,500/μL and platelet count > 100,000/μL, total bilirubin within normal range, AST and ALT < 3× the upper limit of normal, creatinine clearance > 45 mL/min, urine protein:creatinine ratio < 1, and no pre-existing peripheral neuropathy of grade 2 or above.

Patients with brain metastasis, tumors that invaded major vessels (eg, the carotid), history of bleeding diathesis, coagulopathy, or gross hemoptysis, or those receiving therapeutic anticoagulation were excluded. After two fatal bleeding events occurred, the protocol was amended to exclude patients with history of tumor-related bleeding in the previous 6 months. Patients with history of hypertension were well controlled on a stable regimen of antihypertensive therapy. Patients with history of active infection or severe comorbidities, such as active cardiac ischemia, congestive heart failure, or severe neurologic or psychiatric disease, were ineligible. The study protocol was approved by the University of Pittsburgh Institutional Review Board, and all patients provided written informed consent before study entry.

Treatment Plan

Pemetrexed 500 mg/m² and bevacizumab 15 mg/kg were given intravenously on the same day, every 21 days, until disease progression. If the first 90-minute bevacizumab infusion was tolerated without adverse events, the second infusion could be delivered over 60 minutes, and all subsequent infusions would be delivered over 30 minutes. All patients received folic acid 1,000 μg once daily orally and vitamin B₁₂ 1,000 μg intramuscularly, every 9 weeks, beginning 1 week before starting treatment. Patients also received dexamethasone 4 mg twice daily orally, 1 day prior, the day of, and 1 day after pemetrexed. Prophylactic administration of granulocyte colony-stimulating factor was not permitted.

Treatment Evaluation

Before initiating treatment, patients were evaluated with a history and physical examination, complete blood count, serum chemistry profile, coagulation tests, urine protein:creatinine ratio on spot urine sample, and ECG. During treatment, clinical and laboratory evaluations were performed before each cycle. Radiologic evaluation by computed tomography and/or magnetic resonance imaging was performed for baseline tumor assessment and after every two cycles of therapy. Toxicities were assessed by using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Gene Polymorphisms in TS, *MTHFR*, and VEGF

DNA was isolated from whole blood samples collected before first drug administration by using commercially available kits. Single nucleotide polymorphisms (SNPs) in TS, *MTHFR*, and VEGF were evaluated. SNPs were detected by using a TaqMan-based SNP genotyping kit (Applied Biosystems, Foster City, CA) run on the ABI Prism 7700 Sequence Detection System v1.7 (Applied Biosystems). The TS gene promoter repeat and SNP (two or three repeats; G>C within second repeat of the 3R allele) polymorphisms were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism–PCR analyses as previously described.²⁸ Positive and negative PCR controls were included with each amplification reaction. Samples were genotyped in a blinded fashion, and an additional 10% of samples were repeated to verify the reproducibility of the assay. All results were interpreted independently by two laboratory observers.

Statistical Methods

The primary end point was TTP, and the study used a one-stage design. Secondary efficacy end points were objective response rate (RR) by RECIST²⁷ and overall survival. TTP was calculated from treatment initiation to disease progression or last follow-up, and overall survival was calculated from treatment initiation to death or last follow-up. A sample size of 40 patients provided 88% power to detect a 2-month improvement in median TTP (ie, from 3.9 months [historical control¹⁴] to 5.9 months) at a significance level of 0.1. TTP for the whole study cohort was estimated by the Kaplan-Meier method with a 90% CI for the median and compared with that of the historical control population with a one-tailed, one-sample log-rank test for which the expected times to progression followed an exponential distribution. TTP and overall survival for genes and polymorphism subsets were estimated by using the Kaplan-Meier method and were compared with a multivariate log-rank permutation test.²⁹ This procedure adjusts the resulting *P* value for multiple testing while fixing any correlations among polymorphisms. If the permutation-adjusted *P* value was < .05, the specific polymorphisms were probed to determine which genotypes contributed to differences in survival.

Table 1. Patient Characteristics (n = 40)

Characteristic	No.	%
Age, years		
Median	59	
Range	36-85	
Sex		
Male	32	80
Female	8	20
ECOG performance status		
0	16	40
1	24	60
Primary site		
Oral cavity	7	18
Oropharynx	21	50
Larynx	4	10
Hypopharynx	2	5
Unknown primary	4	10
Other	2	5
Prior chemotherapy as part of curative treatment	29	72.5
Prior EGFR inhibitors	8	20*
Locally recurrent	22	55
Metastatic	32	80†

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor.
 *Three patients within 6 months.
 †Fourteen patients had both locally recurrent and metastatic disease.

Hardy-Weinberg equilibrium for gene polymorphisms was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected frequencies.

RESULTS

Forty patients were enrolled in three participating centers, including affiliated community hospitals. Baseline characteristics are provided in Table 1. Twenty-six of 29 patients who had received chemotherapy as part of previous potentially curative therapy received a platinum-based regimen. The median number of cycles of therapy delivered was six (range, one to 30). Two patients had dose reductions of pemetrexed for grade 3 toxicities, and 10 patients had at least one bevacizumab dose delayed or omitted for either toxicities or unrelated events (no dose reductions of bevacizumab were allowed).

Efficacy

The median TTP was 5 months (90% CI, 4 to 7 months; Fig 1). Thus, the null hypothesis of a median TTP of 3.9 months was rejected ($P = .02$). Eight patients were alive at the time of the analysis, and these patients had a median follow-up of 10 months (range, 3 to 33 months). The median overall survival was 11.3 months (90% CI, 8.7 to 16.8 months; Fig 1). Thirty-seven of 40 patients were evaluable for response. Three patients had no repeat tumor assessments performed because they were removed from the study early, after one to two cycles, because of toxicities. The objective RR was 30% (90% CI, 17% to 42%; nine partial and two complete responses), and the disease control rate was 86% (90% CI, 77% to 96%; Table 2). Two partial responses and one complete response were observed in the 11

Fig 1. Kaplan-Meier estimates of (A) time-to-progression and (B) overall survival (solid line) with 90% CIs (dotted line). The median time-to-progression was 5 months, and the median overall survival was 11.3 months.

chemotherapy-naïve patients; there was no difference in RR or survival between these patients and the rest of the patients.

Safety

All patients were evaluable for toxicity. Grade 3 to 5 toxicities reported during the study are listed in Table 3. Six patients (15%) had grade 3 to 5 bleeding events, two of which were grade 5. One patient suffered fatal tracheal bleeding; this patient had a prior history of

Table 2. Objective Response

Response Type	No. of Patients	% of Evaluable Patients (n = 37)	% of All Patients (n = 40)
Objective response	11	30	27.5
Complete response	2	5	5
Partial response	9	24	22.5
Stable disease	21	57	52.5
Disease control (objective response and stable disease)	32	86	80
Progressive disease	5	13	12.5
Nonevaluable	3	—	7.5

Table 3. Grade 3 to 5 Toxicities (n = 40)

Toxicity	Grade					Total	
	3	4	5	6	7		
Anemia	2	5	0	0	0	2	5
Neutropenia	4	10	0	0	0	4	10
Infection	4	10	0	1	2.5*	5	12.5
Fatigue	2	5	0	0	0	2	5
Hypokalemia	1	2.5	1	2.5	0	2	5
Hyponatremia	2	5	0	0	0	2	5
Anorexia	1	2.5	0	0	0	1	2.5
Mucositis	1	2.5	0	0	0	1	2.5
AST increase	1	2.5	0	0	0	1	2.5
Edema	1	2.5	0	0	0	1	2.5
Hypertension	1	2.5	0	0	0	1	2.5
Bleeding	4	10	0	2	5	6	15

*One patient died of sepsis probably associated with fistula after receiving eight cycles.

bloody tracheal secretions associated with tumor invading the trachea that had previously responded to reirradiation. Another fatal event occurred after rechallenging with bevacizumab after recurrent tumor-related bleeding. Two grade 2 and six grade 1 bleeding events were also noted. Other serious toxicities in 10% or more of patients included neutropenia (10%) and infection (12.5%). One patient died of sepsis that was probably related to fistula formation after receiving eight cycles of therapy.

Association of Polymorphisms With Outcome

DNA samples from a total of 28 patients were available and genotyped. No clinical or pathologic differences were detected between the 28 patients with and the 12 patients without genotyping. The frequencies of the *MTHFR*, *TS*, and *VEGF* polymorphisms are provided in Table 4. The genotype frequencies were in Hardy-Weinberg equilibrium, except for the *VEGF* rs699947 ($P < .03$). Although this indicates a possible genotyping assay bias, the assay performance was verified in a separate study of healthy controls in which Hardy-Weinberg equilibrium was satisfied.

Overall survival differed by the *MTHFR* A1298C SNP ($P = .034$; Fig 2). The median OS for patients with AC/CC was 32 months compared with 11 months for AA ($P = .004$). However, this SNP was apparently not associated with TTP ($P = .174$). The median TTP for

Fig 2. Overall survival according to *MTHFR* A1298C polymorphisms ($P = .034$, log-rank test adjusted).

AC/CC and AA patients was 6.9 months and 4.1 months, respectively ($P = .063$).

Although *VEGF* G634C SNP also yielded a statistically significant association with overall survival and TTP, only one patient exhibited the *VEGF* G634C GG genotype, and outcomes by GC and CC genotypes were similar. There were no other significant associations between genotypes and efficacy or toxicity for the other SNPs evaluated in this study.

DISCUSSION

The investigation of novel agents in SCCHN is of major interest. Although the incorporation of cetuximab into platinum and FU has improved overall survival and other efficacy parameters in recurrent or metastatic SCCHN,⁹ prognosis remains poor, and treatment-related toxicities are frequently considerable. We studied a novel regimen with pemetrexed and bevacizumab in patients with recurrent or metastatic SCCHN. Our central hypothesis was that bevacizumab can potentiate the antitumor activity of pemetrexed as has been shown for other chemotherapy agents in solid malignancies. Using a non-platinum doublet, we report efficacy results (median TTP of 5 months and median overall survival of 11.3 months) that are at least comparable with those achieved with the combination of platinum, FU, and cetuximab.⁹ However, we acknowledge the limitations of a comparison between results of a single-arm phase II trial and a large multicenter phase III trial. Furthermore, it is of interest that although pemetrexed has poor activity in SCC of the lung, it appears to be active in SCCHN. Nevertheless, pemetrexed did not improve survival ($P = .08$) or progression-free survival ($P = .166$) when added to cisplatin in a phase III trial in recurrent or metastatic SCCHN.¹⁵ Subset analysis demonstrated survival benefit in patients with good performance status or oropharyngeal primaries.¹⁵ In our study, half the patients had oropharyngeal primaries, which is a favorable prognostic factor in recurrent or metastatic SCCHN³ and may have favorably influenced efficacy results.

Two other clinical trials, one presented in abstract form,^{30,31} have investigated the combination of an epidermal growth factor receptor inhibitor with bevacizumab. A phase I/II trial of erlotinib and bevacizumab in patients with recurrent or metastatic SCCHN showed

Table 4. Frequency of *TS*, *MTHFR*, and *VEGF* polymorphisms (n = 28)

Gene Name	Variant	Allele Frequency*	Hardy-Weinberg Equilibrium <i>P</i>
<i>MTHFR</i>	rs1801133	16/8/4	.11
<i>MTHFR</i>	rs1801131	13/12/3	.93
<i>MTHFR</i>	rs2274976	23/5/0	.6
<i>TS</i>	Repeat	11/14/3	.64
<i>TS</i>	6 bp deletion	14/9/5	.13
<i>VEGF</i>	rs699947	7/19/2	.03
<i>VEGF</i>	rs2010963	10/17/1	.06
<i>VEGF</i>	rs3025039	25/3/0	.76

Abbreviation: bp, base pair.

*Number of common allele homozygotes/heterozygotes/minor allele homozygotes.

promising results; median overall survival was 7.1 months and progression-free survival was 4.1 months.³⁰ Three bleeding events (one of which was fatal) were reported in 48 patients. In our study, bleeding events were frequent, and six patients had serious bleeding of grade 3 or higher. However, hemorrhagic complications were rare in a phase II trial with cetuximab and bevacizumab in patients with recurrent or metastatic SCCHN.³¹ In patients with SCC of the lung, bevacizumab results in unacceptable rates of serious and fatal bleeding complications, and thus bevacizumab use has been restricted to non-squamous non-small-cell lung cancer.³² We observed frequent serious bleeding events in patients treated with pemetrexed and bevacizumab in our study (15%), including two deaths, which is a potential concern for the safety of the regimen. However, this rate of serious bleeding events may not be prohibitive for further evaluation of the pemetrexed/bevacizumab combination in SCCHN, especially given the rarity of other serious adverse events. Hemorrhagic complications are not uncommon in the natural history of SCCHN, and it is sometimes difficult to discern the contribution of study drugs to these events. The application of careful eligibility and re-treatment criteria regarding tumor-related bleeding can potentially reduce the incidence of these events. Ultimately, a randomized comparison of chemotherapy with or without bevacizumab will be able to accurately evaluate the added toxicities and benefits with bevacizumab in recurrent or metastatic SCCHN. A phase III trial with cisplatin-based chemotherapy with or without bevacizumab is currently ongoing in the ECOG E1305 trial.

One challenge in the incorporation of new agents into cancer therapeutics has been the identification of reliable and easily reproducible predictive factors. In this study, we conducted an exploratory analysis of *MTHFR*, *TS*, and *VEGF* polymorphisms and their association with efficacy of bevacizumab and pemetrexed combination. Altered reduced folate pools that relate to *MTHFR* activity may modulate response to antifolates. Here, we observed a significant association between the *MTHFR* A1298C allele and survival, with homozygous carriers of the common allele (AA) having reduced survival ($P = .034$). Previously, *MTHFR* SNPs have been found to be associated with survival in patients with colon cancer,^{33,34} breast cancer,³⁵ non-small-cell lung cancer,³⁶ and other malignant tumors²⁵; however, some of these studies reported a significant association of the reduced activity of the variant C allele with poorer prognosis. Prospective clinical studies should be performed to validate *MTHFR* polymorphisms as a predictive or prognostic marker in patients with SCCHN who have been treated with pemetrexed. Biomarkers for antiangiogenesis therapy in SCCHN are also under investigation.³⁰ In our study,

we examined several *VEGF* SNPs, some of which were previously shown to relate to the outcome of patients with breast cancer who had been treated with bevacizumab-containing therapy²⁶; however, our exploratory analysis did not reveal any clinically significant associations.

In conclusion, the addition of bevacizumab to pemetrexed showed promising efficacy results in recurrent or metastatic SCCHN. Bleeding events were frequent in this study, but some may have been related to the natural history of the disease. The use of *MTHFR* polymorphisms for treatment individualization warrants further investigation in this setting. Bevacizumab-containing regimens should be further investigated in SCCHN.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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