

Prognostic significance of p16 in locoregionally advanced head and neck cancer treated with concurrent 5-fluorouracil, hydroxyurea, cetuximab and intensity-modulated radiation therapy

CHARLES C.L. TONG^{1,3*}, K.H. VINCENT LAU^{1*}, MICHAEL RIVERA², DAVID CANNAN³, JULIO AGUIRRE-GHISO³, ANDREW G. SIKORA³, VISHAL GUPTA¹, KEVIN FORSYTHE¹, ERIC C. KO¹, KRZYSZTOF MISIUKIEWICZ⁴, VIVEK GURUDUTT³, MARITA S. TENG³, STUART H. PACKER⁴, ERIC M. GENDEN³ and JOHNNY KAO⁵

Departments of ¹Radiation Oncology, ²Pathology, ³Otolaryngology-Head and Neck Surgery, and ⁴Medical Oncology, Mount Sinai School of Medicine, New York, NY; ⁵Florida Radiation Oncology Group, Brandon, FL, USA

Received November 30, 2011; Accepted January 4, 2012

DOI: 10.3892/or.2012.1679

Abstract. A phase II trial was conducted to evaluate the tolerability and efficacy of incorporating cetuximab and simultaneous integrated boost intensity-modulated radiation therapy (SIB-IMRT) into a well-described 5-fluorouracil (5-FU) and hydroxyurea (HU)-based chemoradiation regimen. Patients with stage IVA-IVB or high-risk stage III squamous cell carcinomas were enrolled. Prior organ-conserving surgery or induction chemotherapy was allowed. IMRT was administered in 1.5 Gy fractions twice daily on days 1-5 of weeks 1, 3, 5, 7 ± 9 for a total dose of 60-73.5 Gy. Concurrent systemic therapy consisted of 5-FU (600 mg/m²), HU (500 mg BID) and cetuximab (250 mg/m²). p16^{INK4A} expression was assessed by immunohistochemistry. From January 2007 to January 2010, 65 patients (61 with stage IV disease; 31 with oropharyngeal primaries) were enrolled. At a median follow-up of 28 months, 2-year locoregional control, distant control, progression-free survival, event-free survival and overall survival were 79, 83, 72, 63 and 80%, respectively. In 48 patients with available pre-treatment tissue, p16 overexpression was associated with significantly increased distant control (p=0.03), progression-free survival (p=0.02), event-free survival (p=0.007) and overall survival (p=0.03). The most common grade 3-4 toxicities were mucositis (46%), leukopenia (18%), anemia (18%) and dermatitis

(17%). Concurrent 5-FU, HU, cetuximab and SIB-IMRT is a highly active regimen, particularly in patients with p16-positive disease.

Introduction

Concurrent chemoradiation is a standard treatment option for most patients with stage III-IVB head and neck cancer (1). Although concurrent high dose cisplatin (100 mg/m² q21 days) is the best studied regimen, accumulating data suggests that either concurrent cetuximab (250 mg/m² q7 days) or 5-fluorouracil (5-FU) and hydroxyurea (HU) are also effective radiosensitizing regimens (2-5). Recent data suggests that adding induction chemotherapy to concurrent chemoradiation contributes to improved distant control and survival (6,7). Although aggressive chemoradiation regimens contributed to promising rates of disease control, significant rates of acute and late toxicity have motivated clinical investigation of biologically targeted agents that hold promise for specific antitumor efficacy with less toxicity than chemotherapy. Epidermal growth factor receptor (EGFR) is overexpressed in 80-90% of patients with head and neck squamous cell carcinoma (HNSCC) and increased EGFR is associated with increased risk of disease progression and mortality following radiotherapy (8). Adding cetuximab to head and neck radiotherapy improves locoregional control and survival without increasing mucosal toxicity. Based on this compelling preclinical and clinical data, there is significant interest in incorporating cetuximab, a monoclonal antibody targeted against EGFR, into existing chemoradiation regimens (8).

To date, several groups have reported phase II trials demonstrating feasibility and encouraging preliminary efficacy data when EGFR inhibitors were added to cisplatin-based chemoradiotherapy regimens (9-12). A recent phase II trial demonstrated promising 4-year progression-free survival of 72% after treatment with induction carboplatin and paclitaxel followed by concurrent 5-FU, HU, gefitinib and hyperfractionated radiotherapy followed by maintenance gefitinib (11). Based on these encouraging data, we hypothesized that cetuximab, a targeted agent approved for use in HNSCC,

Correspondence to: Dr Charles C.L. Tong, Department of Radiation Oncology, and Department of Otolaryngology-Head and Neck Surgery, Mount Sinai School of Medicine, 1 Gustave Levy Place, Box 1189, New York, NY 10029, USA
E-mail: charles.tong@mssm.edu

*Contributed equally

Key words: cetuximab, chemoradiation, intensity modulated radiation therapy, head and neck cancer, epidermal growth factor receptor

would further improve outcomes without increasing toxicity. We have previously reported preliminary efficacy results (13).

Despite the high rate of EGFR overexpression, only ~10% of patients respond to EGFR inhibitors, and no prognostic factor can predict the response to EGFR-based therapy in HNSCC (8,14,15). Approximately 25% of head and neck squamous cell carcinoma (HNSCC) cases worldwide are associated with high-risk human papillomaviruses (HPV) 16, 18, 31, 33 and 35, with HPV-16 accounting for ≥90% of HPV-positive oropharyngeal cancer (16). Although data on the HPV infection status was not available in the phase III trial reported by Bonner *et al*, analysis of the Forrest plots suggests that the benefit of cetuximab occurs predominantly in younger patients with small oropharyngeal primary tumors with advanced nodal disease (3). This patient population has been strongly associated with high-risk HPV infection (17). p16^{INK4A} is a validated surrogate marker for overexpression of HPV-16 and has been extensively investigated as a prognostic factor for head and neck cancer patients treated with radiation or chemoradiation (18-20). Therefore, we evaluated a second hypothesis that p16-positive tumors are more likely to respond to cetuximab-based chemoradiation than p16-negative tumors.

Patients and methods

Ethics statement. Two phase II trials (MSSM 06-1155; NCT00462735) were approved by the Mount Sinai School of Medicine Institutional Review Board. All patients signed written informed consent.

Study design and eligibility. All patients had histologically confirmed head and neck squamous cell carcinoma or poorly differentiated carcinoma. Eligible patients had stage IVA-IVB disease according to the 6th edition of the AJCC staging guidelines, or high-risk stage III disease, defined as base of tongue or hypopharynx primary or major pathological risk factors (microscopic positive margins or extracapsular extension). Patients were required to have an ECOG performance status of ≤2 and adequate bone marrow, kidney and liver function based on pre-treatment laboratory evaluation. All cases were reviewed at a multidisciplinary conference attended by representatives from head and neck surgery, radiation oncology, medical oncology, palliative care, social work and nutrition. Organ preserving surgery, defined as selective neck dissection or preservation of tongue, larynx, orbit, mandible or facial nerve and/or induction chemotherapy was allowed prior to registration on the protocol. Patients who received prior head and neck radiation were ineligible (Fig. 1).

Initial staging procedures included history and physical, nasolaryngoscopy and biopsy, dental evaluation, head and neck computed tomography (CT) and chest CT with or without positron emission tomography (PET). Prophylactic feeding tubes were strongly recommended for patients with oral cavity involvement, massive tumors, advanced age or limited physiological reserve. Patients were followed prospectively by the multidisciplinary team usually with PET/CT-based follow-up (21).

Week on/week off (WO/WO) chemoradiotherapy. Patients received concurrent chemoradiotherapy consisting of continuous infusion 5-FU at 600 mg/m²/day x 120 h (days 1-5) and

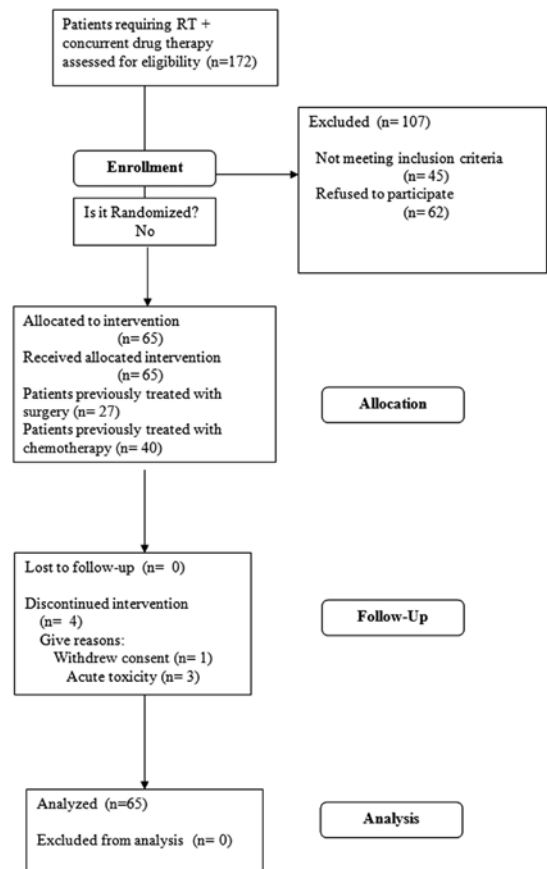


Figure 1. CONSORT flowchart.

HU 500 mg orally q12 hours (days 1-5) with the morning dose administered 2 h before radiation and cetuximab 250 mg/m² (day 1) on 14-day treatment cycles. Patients with gross disease received 5 cycles of chemoradiation, whereas post-surgical patients with microscopic disease received 4 cycles. Treatment was administered on an inpatient basis. For the first 33 patients, no anticancer therapy was administered on days 6-14. Based on the favorable toxicity profile seen in the initial cohort, the protocol was modified to add cetuximab 250 mg/m² on day 9 for the remaining 32 patients (13). Concurrent chemoradiotherapy was withheld only for absolute neutrophil count (ANC) <500/μl, fever, infection or patient refusal but not for grade ≤3 mucositis or dermatitis. The cetuximab dose was reduced by 20% for creatinine clearance <50 ml/min or grade ≥3 asthenia or nausea/vomiting attributed to cetuximab. Grade ≥3 leukopenia or platelet resulted in a reduction in HU in subsequent cycles. Filgrastim 5 μg/kg was given by daily subcutaneous injection on days 6 through 12 following prior grade ≥3 neutropenia.

Radiotherapy was administered at 1.5 Gy per fraction twice daily with fractions separated by at least 6 h on days 1-5 on an alternating weekly schedule. Intensity-modulated radiation therapy (IMRT) was utilized for all patients. Patients underwent CT simulation, usually with fusion of PET or MRI imaging to assist with target delineation. When applicable, pre-induction chemotherapy tumor volumes were targeted.

Gross tumor volumes received 72 to 73.5 Gy, microscopic positive margins received 66 Gy, high-risk microscopic

Table I. Patient, tumor and treatment characteristics.

Characteristic	No. (%)
Induction chemotherapy	
Yes	40 (62)
No	25 (38)
Surgery prior to chemoRT	
Yes	27 (42)
No	38 (58)
Cetuximab schedule	
Biweekly	33 (51)
Weekly	32 (49)
Median age; range	59 (18-80)
Gender	
Male	50 (77)
Female	15 (23)
Performance status	
0	20 (31)
1	33 (51)
2	12 (18)
Race	
White	49 (75)
Black	7 (11)
Hispanic	7 (11)
Asian	2 (3)
Smoking history	
None	14 (22)
Cigar, pipe, marijuana or betel nut only	5 (8)
≤10 pack years	12 (18)
10.1-40 pack years	19 (29)
≥40 pack years	15 (23)
Primary site	
Sinonasal	3 (5)
Nasopharynx	5 (8)
Oropharynx	31 (48)
Oral cavity	8 (12)
Salivary gland	2 (3)
Larynx	5 (8)
Hypopharynx	7 (11)
Unknown primary	4 (6)
AJCC stage	
III	4 (6)
IVA	50 (77)
IVB	11 (17)
T stage	
T 0-2	29 (45)
T 3-4	36 (55)
N stage	
N0	9 (14)
N1	9 (14)
N2	42 (65)
N3	5 (8)
p16 status	
Negative	26 (40)
Positive w/ ≤10 pack years	12 (28)
Positive w/ >10 pack years	10 (15)
Unknown	17 (26)

disease (resected tumor bed or first echelon of uninvolved nodal stations) received 54 to 63 Gy and low-risk microscopic disease (low-risk nodal stations) received 43.2 to 48 Gy. The microscopic volumes were treated with a simultaneous integrated boost plan. Radiation dose constraints were described previously (13). A separate cone down was performed for patients with gross disease. Patients received the lower dose levels if they achieved a partial or complete response to induction chemotherapy. The median dose was 72 Gy (range 13.5 to 73.5 Gy). The median duration of the treatment was 60 days (range 5 to 87 days).

Induction chemotherapy. Induction chemotherapy, given off protocol, was administered to selected patients at the discretion of the treating medical oncologist (Table I). The most common indication for induction chemotherapy was high-risk of distant failure due to advanced N2b to N3 nodal disease. Measurable disease was not considered a requirement for induction chemotherapy. Docetaxel (75 mg/m²), cisplatin (75 mg/m²) and 5-FU (750 mg/m² x 5 days) q3 weeks for 2 cycles was given to 40 patients (62%).

Surgery. Organ-conserving surgery was performed in 27 patients (42%) prior to chemoradiation (Table I). This included 16 patients treated with resection of the primary site and selective neck dissection, 3 patients with resection of the primary site alone and 8 patients with neck dissection alone. Adverse pathology included 14 patients with microscopic positive margins, 14 patients with perineural invasion, 9 patients with lymphovascular invasion, 15 patients with multiple pathologically positive nodes and 18 patients with extracapsular extension. Selective neck dissection after complete response of N2-3 nodal disease following chemoradiation was performed in 6 patients.

p16^{INK4A} immunohistochemistry. Tumor blocks were available in 48 pre-treatment patients (74%). Briefly, tissues were fixed in 10% formalin, embedded in paraffin and 5 μm sections were adhered to charged glass slides. Slides were deparaffinized in a 60°C oven for 30 min prior to xylene bath, and rehydrated with ethyl alcohol. Prior to immunostaining, epitope retrieval was achieved by incubating slides in retrieval solution placed in a 97°C water bath for 10 min and allowed to cool to room temperature for 20 min. Endogenous peroxidase activity was blocked using peroxidase-blocking reagent followed by application of the primary mouse anti-human p16^{INK4A} antibody (mtm Laboratories, Westborough, MA) for 30 min at room temperature. The visualization reagent containing goat anti-mouse secondary antibody was applied for 30 min at room temperature followed by DAB chromogen for 1 min. Counterstaining was achieved using Mayer's hematoxylin. After dehydration and mounting, slides were scored for p16 staining as negative (Fig. 3A), 1⁺, 2⁺ and 3⁺ by a single pathologist (M.R.) with subspecialty training in head and neck cancer pathology who was blinded to patient outcome. Scores of 2⁺ to 3⁺ were considered positive (Fig. 3B). Since p16 staining is routinely used in our clinical practice, previously scored patients with p16-positive and negative tumors were used as controls.

Treatment evaluation and statistical considerations. The primary endpoint was progression-free survival, measured

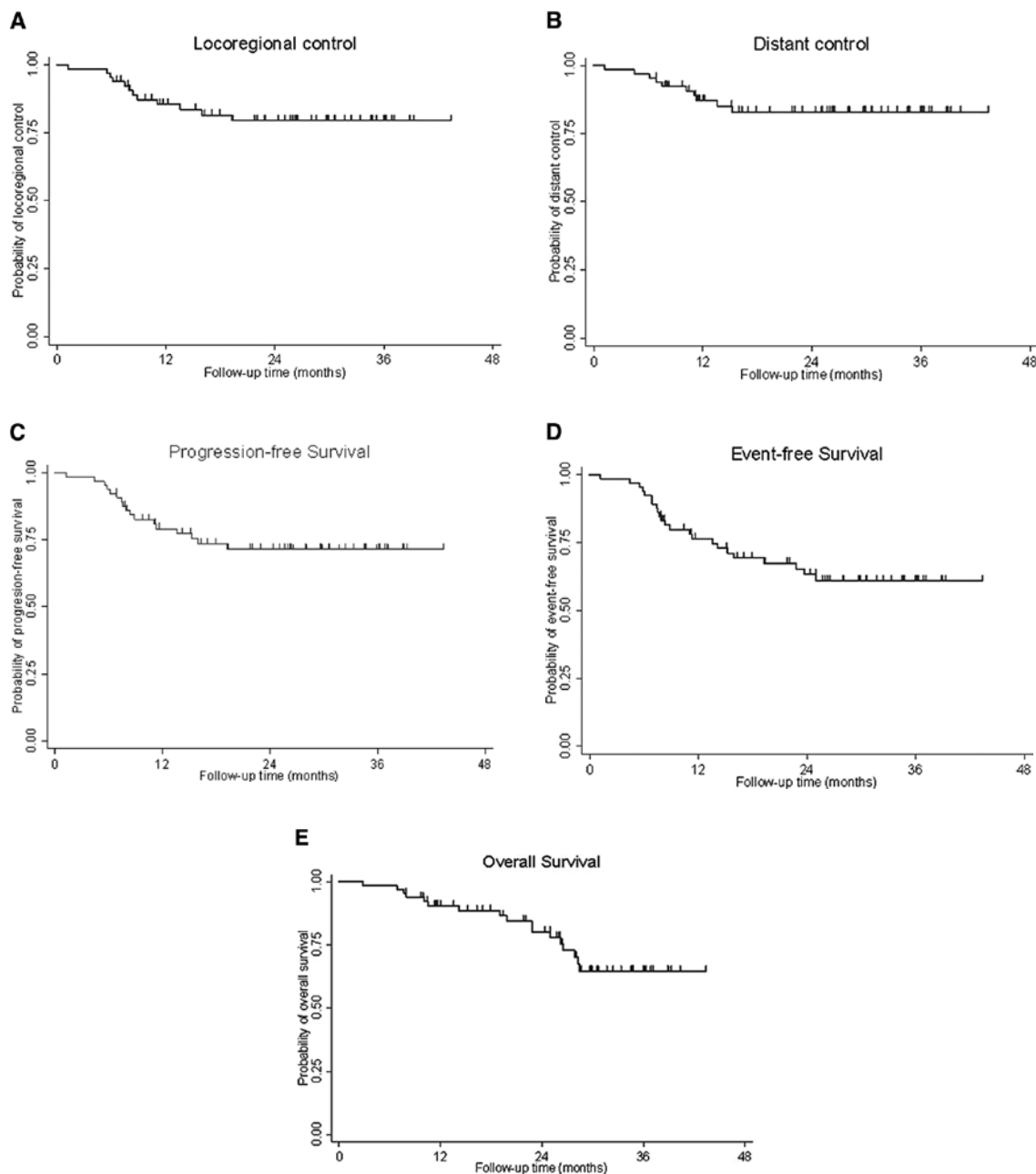


Figure 2. Kaplan-Meier survival curves: (A) locoregional control, (B) distant control, (C) progression-free survival, (D) event-free survival, (E) overall survival.

as time from the initiation of non-surgical treatment until last follow-up or disease progression using intent to treat principles. Failures were scored as local, regional or distant. Event-free survival is a composite endpoint that includes disease progression, second primary tumor or death. Survival curves were calculated using Kaplan-Meier curves using Stata 9.1. Differences in survival curves were calculated by using the log-rank test. Univariate and multivariable analyses of patient and disease factors include age, race, smoking history, gender, performance status, stage, tumor site, p16, surgery, induction chemotherapy and frequency of cetuximab.

Results

Patient characteristics. From January 2007 to January 2010, 65 patients were enrolled (Table I). Median follow-up for

surviving patients was 28 months (range 8-43 months). The patient characteristics are summarized in Table I. The median age was 59 years (range 18-80). Sixty-one patients (94%) had stage IV disease and 4 patients (6%) had stage III disease, including 7 patients with radiographic evidence of progression following either surgery or induction chemotherapy prior to starting chemoradiation. Thirty-six patients (55%) had T3-4 primary tumors and 18 patients (27%) had N2-3 nodal disease (Table I).

Survival and patterns of failure. The 2-year locoregional control, distant control, progression-free survival, event-free survival and overall survival were 79, 83, 72, 63 and 80%, respectively (Fig. 2). The 2-year local control rate was 85% and the 2-year regional control rate was 82%. At the time this manuscript was prepared, 17 patients (26%) had experienced

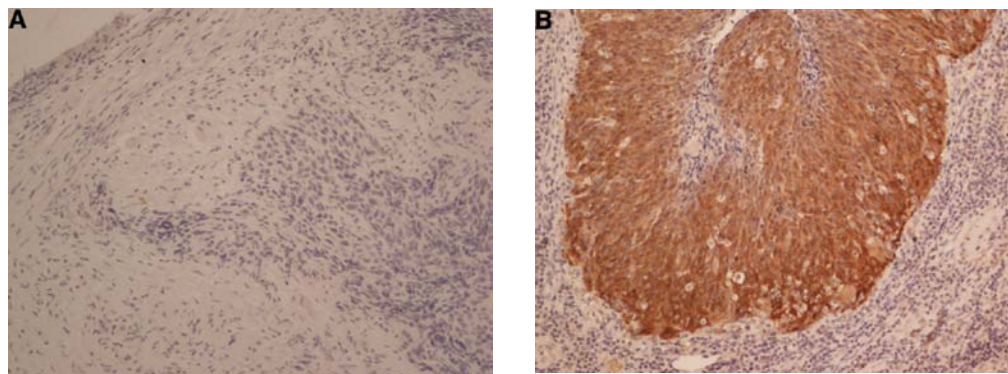


Figure 3. p16 immunostaining of pre-treatment tumor specimens at 20x magnification. (A) Note irregular infiltrates of tumor cells with absence of p16 staining. (B) Note intense tumor-specific staining with nuclear enhancement and absence of staining in adjacent stroma.

Table II. Percentage of patients (n=65) with acute toxicity grade during concurrent chemoradiotherapy.

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Mucositis	11	40	46	2
Dermatitis	30	52	17	0
Pain	6	43	49	0
ANC	29	23	9	0
WBC	28	46	18	0
Hgb	35	43	18	0
Plt	31	0	2	0
Nausea/vomiting	8	3	0	0
Infection	2	6	2	3
Xerostomia	44	48	0	0
Sialadenitis	0	5	0	0
Dry eye	0	3	0	0

ANC, absolute neutrophil count; WBC, white blood cells; Hgb, hemoglobin; Plt, platelets.

disease recurrence. Patterns of failure were locoregional only in 7, distant only in 5 and both locoregional and distant in 5. Additionally, four patients developed second primary tumors; 12 patients had died of recurrent disease, 2 had died of second primary tumors and 2 patients had died of comorbid illnesses.

Effect of p16 status on disease control. Increased p16 expression was strongly associated with improved 2-year overall survival (94.4 vs. 69.5%; $p=0.03$), progression-free survival (86.4 vs. 54.3%; $p=0.02$), event-free survival (86.3 vs. 41.5%; $p=0.007$), locoregional control (90.9 vs. 64.2%; $p=0.05$) and distant control (95.5 vs. 71.5%; $p=0.03$) on univariate analysis (Fig. 4). Patients with p16-positive tumors with a minimal smoking history (≤ 10 pack years) had a significantly improved 2-year event-free survival (87.5 vs. 70.0%; $p=0.02$) compared to p16-positive smokers (>10 pack years). Multivariable analysis demonstrated that p16 status, stratified by smoking status, was the only significant predictor of event-free survival [hazard ratio (HR), 0.43; $p=0.04$] while there was a marginal effect for AJCC stage (HR, 4.1; $p=0.09$) (Table III).

Table III. Multivariable analyses.

Factor	HR	95% CI	p
Age (continuous)	1.003	0.95-1.05	0.89
Gender (female vs. male)	0.65	0.20-2.1	0.48
Performance status (0 vs. 1 vs. 2)	1.70	0.68-4.27	0.26
Race (black vs. white)	2.14	0.72-6.41	0.17
p16 status (p16 ⁻ , p16 ⁺ /smoker, p16 ⁺ /nonsmoker)	0.43	0.19-0.95	0.04
Primary site (oropharynx vs. non-oropharynx)	0.78	0.23-2.61	0.69
Stage (III, IVA, IVB)	4.12	0.82-20.80	0.09
Induction (yes vs. no)	0.33	0.08-1.40	0.13
Surgery (yes vs. no)	0.73	0.24-2.21	0.58
Cetuximab schedule (biweekly vs. weekly)	0.73	0.22-2.47	0.62

HR, hazard ratio; CI, confidence interval.

Effect of site-specific tumors and p16 status on disease control. Of the 48 patients with pre-treatment tissues available for p16 staining, 22 (46%) were tumors from the oropharynx and 26 (54%) were tumors from non-oropharyngeal sites. Increased p16 expression (n=15) among the oropharyngeal group was strongly associated with overall survival (93 vs. 43%; $p=0.015$), locoregional control (100 vs. 43%; $p=0.001$), distant control (93 vs. 43%; $p=0.028$), progression-free survival (93 vs. 43%; $p=0.005$), and event-free survival (93 vs. 14%; $p=0.001$). Patients with p16-positive non-oropharyngeal tumors (n=7) did not demonstrate statistically significant benefit in similar endpoints.

Adverse events. A percutaneous gastrostomy (PEG) tube was placed in 18% of patients before initiation of radiation while 52% of the patients ultimately required PEG, total parenteral nutrition or a jejunostomy tube during treatment. The median weight loss was 9.5% (range 0-17.4%). The most common grade ≥ 3 acute toxicities were mucositis (48%), anemia (18%), leukopenia (18%), dermatitis (17%) and neutropenia (9%) (Table II). Other frequent acute low-grade treatment-related toxicities included fatigue,

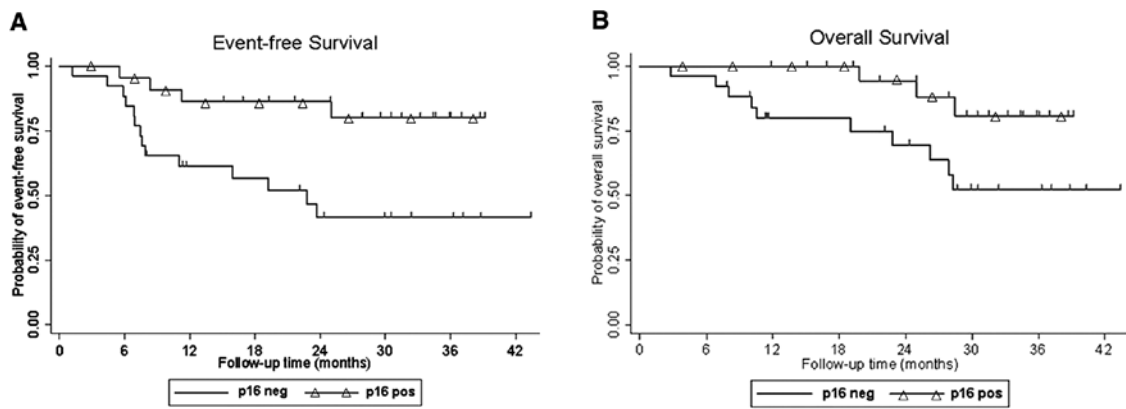


Figure 4. Kaplan-Meier survival curves stratified by p16 status: (A) event free survival, (B) overall survival.

xerostomia, taste changes and thrush. Although there were no grade 5 events or neutropenic infections, two patients developed grade 4 non-neutropenic aspiration pneumonia requiring intensive care unit (ICU) care. Both patients were ultimately moved from the ICU but discontinued radiation at doses of 13.5 Gy and 33 Gy, respectively. Therefore, 97% of patients were able to complete $\geq 95\%$ of the planned radiation dose. Other protocol deviations included 4 patients that had an unplanned treatment break of ≥ 7 days due to toxicity or non-compliance and 1 patient who withdrew consent during chemoradiation and requested an alternative chemoradiation regimen.

Excluding patients with nutritional problems related to recurrent disease, only 2 patients required prolonged use of a feeding tube ≥ 1 year. Other grade 2-3 late toxicities included xerostomia (n=12), bone necrosis (n=3), soft tissue necrosis or ulceration (n=3), esophageal stricture (n=3), skin ulceration (n=1), trismus (n=1) and dysphagia to solid foods (n=5). Two of these patients required reconstructive surgery while the other adverse events were treated conservatively.

Discussion

This report of a single institution phase II trial demonstrates that concurrent 5-FU, HU, cetuximab and hyperfractionated IMRT is a feasible and active regimen in stage III-IVB HNSCC. Despite this complex regimen, often preceded by induction chemotherapy or surgery, approximately 30% of treated patients progressed. The 2-year locoregional control was 79% while the 2-year distant control was 83%. Prior phase II trials performed at the University of Chicago consisting of induction chemotherapy followed by 5-FU, hydroxyurea, paclitaxel and hyperfractionated RT demonstrated rates of locoregional control and distant control in the 90% range (22-24). Although a randomized trial is necessary to test this hypothesis, these data suggest that cetuximab-based chemoradiation may be slightly less effective than an intensive paclitaxel or cisplatin-based regimen. Overall, progression-free survival following treatment with concurrent 5-FU, hydroxyurea, cetuximab and hyperfractionated IMRT compares favorably with other published chemoradiation regimens (7,8,25,26).

Although 5-FU, HU, paclitaxel and hyperfractionated radiation demonstrates robust antitumor activity, a significant subset of patients treated with this intensive regimen succumbed to death

attributed to toxicity or comorbid illness (27). Numerous strategies to reduce acute and chronic toxicity have been examined, including using IMRT in lieu of conventional radiation, reducing radiation doses in responders to induction chemotherapy and using EGFR-inhibitors instead of paclitaxel (11,28,29). Although the marginal benefit of each individual strategy was difficult to detect, implementing all three approaches together in this trial appeared to reduce the incidence of treatment-related toxicity and death compared to prior studies (13). A key advantage of 5-FU, hydroxyurea, cetuximab and hyperfractionated IMRT are low rates of myelosuppression and neutropenic infection. A clear disadvantage is the significant logistical challenges associated with inpatient administration of chemoradiation that includes twice-daily IMRT. As a result, identifying a cohort that benefits from this non-standard but effective strategy for locally advanced HNSCC would guide clinical decisions and future research directions.

Human papillomavirus-16 is an important etiologic and prognostic factor for HNSCC (17). HPV-related tumors have significantly improved locoregional control and survival when treated with radiation alone, concurrent chemoradiation, concurrent chemotherapy and accelerated radiation, induction chemotherapy followed by chemoradiation or surgery (19,25,30,31). To date, it is not known whether HPV-related tumors are more likely to respond to EGFR inhibitors (15). In this study of patients treated with cetuximab-based chemoradiation, patients with p16-positive tumors had significantly improved overall survival, event-free survival, progression-free survival, locoregional control and distant control compared to p16-negative tumors. Although not definitive, these data suggest p16-positive oropharyngeal tumors respond much more favorably to cetuximab-based chemoradiation. More effective treatment for the HPV-negative cohort represents a high priority unmet need for future research. To date, this cohort appears to be refractory to various treatment intensification strategies including sequential chemotherapy followed by concurrent chemotherapy and accelerated fractionation radiation enhanced by concurrent chemotherapy (25,30).

Preclinical data suggests that cetuximab-mediated enhancement of radiotherapy may be dose-dependent (32). Interestingly, there was no significant difference in the outcome detected in this study whether or not patients received additional cetuximab during non-radiation weeks. In the study

of Bonner *et al*, concurrent cetuximab and radiation improved locoregional control compared to radiation alone without improving distant control (8). Taken together, the data suggest that short course cetuximab given during radiotherapy is ineffective at targeting micrometastases. The role of maintenance treatment with EGFR-inhibitors to improve progression-free survival is under active investigation (11).

In conclusion, concurrent 5-FU, hydroxyurea, cetuximab and hyperfractionated IMRT is an effective approach for the treatment of locoregionally advanced HNSCC. Despite aggressive treatment, patients with p16-negative tumors continue to have a poor prognosis.

Acknowledgements

This study was funded in part by the Mount Sinai School of Medicine. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. J.K. received research funding from OSI Pharmaceuticals. S.P. and J.K. were members of the Speakers' Bureau for Sanofi-Aventis. The authors have declared that no competing interests exist.

References

- Haddad RI and Shin DM: Recent advances in head and neck cancer. *N Engl J Med* 359: 1143-1154, 2008.
- Adelstein DJ, Li Y, Adams GL, *et al*: An intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiotherapy in patients with unresectable squamous cell head and neck cancer. *J Clin Oncol* 21: 92-98, 2003.
- Bonner JA, Harari PM, Giralt J, *et al*: Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol* 11: 21-28, 2010.
- Forastiere AA, Goepfert H, Maor M, *et al*: Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. *N Engl J Med* 349: 2091-2098, 2003.
- Garden AS, Harris J, Vokes EE, *et al*: Preliminary results of Radiation Therapy Oncology Group 97-03: a randomized phase II trial of concurrent radiation and chemotherapy for advanced squamous cell carcinomas of the head and neck. *J Clin Oncol* 22: 2856-2864, 2004.
- Brockstein B, Haraf DJ, Rademaker AW, *et al*: Patterns of failure, prognostic factors and survival in locoregionally advanced head and neck cancer treated with concomitant chemoradiotherapy: a 9-year, 337-patient, multi-institutional experience. *Ann Oncol* 15: 1179-1186, 2004.
- Posner MR, Hershock DM, Blajman CR, *et al*: Cisplatin and fluorouracil alone or with docetaxel in head and neck cancer. *N Engl J Med* 357: 1705-1715, 2007.
- Bonner JA, Harari PM, Giralt J, *et al*: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 354: 567-578, 2006.
- Merlano M, Russi E, Benasso M, *et al*: Cisplatin-based chemoradiation plus cetuximab in locally advanced head and neck cancer: a phase II clinical study. *Ann Oncol* 22: 712-717, 2010.
- Pfister DG, Su YB, Kraus DH, *et al*: Concurrent cetuximab, cisplatin, and concomitant boost radiotherapy for locoregionally advanced, squamous cell head and neck cancer: a pilot phase II study of a new combined-modality paradigm. *J Clin Oncol* 24: 1072-1078, 2006.
- Cohen EE, Haraf DJ, Kunnavakkam R, *et al*: Epidermal growth factor receptor inhibitor gefitinib added to chemoradiotherapy in locally advanced head and neck cancer. *J Clin Oncol* 28: 3336-3343, 2010.
- Harrington KJ, El-Hariry IA, Holford CS, *et al*: Phase I study of lapatinib in combination with chemoradiation in patients with locally advanced squamous cell carcinoma of the head and neck. *J Clin Oncol* 27: 1100-1107, 2009.
- Kao J, Genden EM, Gupta V, *et al*: Phase 2 trial of concurrent 5-fluorouracil, hydroxyurea, cetuximab, and hyperfractionated intensity-modulated radiation therapy for locally advanced head and neck cancer. *Cancer* 117: 318-326, 2011.
- Cohen EE, Rosen F, Stadler WM, *et al*: Phase II trial of ZD1839 in recurrent or metastatic squamous cell carcinoma of the head and neck. *J Clin Oncol* 21: 1980-1987, 2003.
- Eriksen JG, Lassen P and Overgaard J: Do all patients with head and neck cancer benefit from radiotherapy and concurrent cetuximab? *Lancet Oncol* 11: 312-313, 2010.
- Kreimer AR, Clifford GM, Boyle P, *et al*: Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 14: 467-475, 2005.
- Gillison ML: Human papillomavirus and prognosis of oropharyngeal squamous cell carcinoma: implications for clinical research in head and neck cancers. *J Clin Oncol* 24: 5623-5625, 2006.
- Shi W, Kato H, Perez-Ordóñez B, *et al*: Comparative prognostic value of HPV16 E6 mRNA compared with in situ hybridization for human oropharyngeal squamous carcinoma. *J Clin Oncol* 27: 6213-6221, 2009.
- Lassen P, Eriksen JG, Hamilton-Dutoit S, *et al*: HPV-associated p16-expression and response to hypoxic modification of radiotherapy in head and neck cancer. *Radiother Oncol* 94: 30-35, 2010.
- Weinberger PM, Yu Z, Haffty BG, *et al*: Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol* 24: 736-747, 2006.
- Kao J, Vu HL, Genden EM, *et al*: The diagnostic and prognostic utility of positron emission tomography/computed tomography-based follow-up after radiotherapy for head and neck cancer. *Cancer* 115: 4586-4594, 2009.
- Michiels S, Le Maitre A, Buyse M, *et al*: Surrogate endpoints for overall survival in locally advanced head and neck cancer: meta-analyses of individual patient data. *Lancet Oncol* 10: 341-350, 2009.
- Haraf DJ, Rosen FR, Stenson K, *et al*: Induction chemotherapy followed by concomitant TFX chemoradiotherapy with reduced dose radiation in advanced head and neck cancer. *Clin Cancer Res* 9: 5936-5943, 2003.
- Vokes EE, Stenson K, Rosen FR, *et al*: Weekly carboplatin and paclitaxel followed by concomitant paclitaxel, fluorouracil, and hydroxyurea chemoradiotherapy: curative and organ-preserving therapy for advanced head and neck cancer. *J Clin Oncol* 21: 320-326, 2003.
- Ang KK, Harris J, Wheeler R, *et al*: Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 363: 24-35, 2010.
- Brizel DM, Albers ME, Fisher SR, *et al*: Hyperfractionated irradiation with or without concurrent chemotherapy for locally advanced head and neck cancer. *N Engl J Med* 338: 1798-1804, 1998.
- Mell LK, Dignam JJ, Salama JK, *et al*: Predictors of competing mortality in advanced head and neck cancer. *J Clin Oncol* 28: 15-20, 2010.
- Milano MT, Vokes EE, Kao J, *et al*: Intensity-modulated radiation therapy in advanced head and neck patients treated with intensive chemoradiotherapy: preliminary experience and future directions. *Int J Oncol* 28: 1141-1151, 2006.
- Salama JK, Stenson KM, Kistner EO, *et al*: Induction chemotherapy and concurrent chemoradiotherapy for locoregionally advanced head and neck cancer: a multi-institutional phase II trial investigating three radiotherapy dose levels. *Ann Oncol* 19: 1787-1794, 2008.
- Fakhry C, Westra WH, Li S, *et al*: Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 100: 261-269, 2008.
- Licitra L, Perrone F, Bossi P, *et al*: High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol* 24: 5630-5636, 2006.
- Milas L, Fang FM, Mason KA, *et al*: Importance of maintenance therapy in C225-induced enhancement of tumor control by fractionated radiation. *Int J Radiat Oncol Biol Phys* 67: 568-572, 2007.