Clinical Cancer Research

Early Tumor Progression Associated with Enhanced EGFR Signaling with Bortezomib, Cetuximab, and Radiotherapy for Head and Neck Cancer

Athanassios Argiris[1], Austin G. Duffy[3], Shivaani Kummar[3], Nicole L. Simone[4], Yoshio Arai[2], Seungwon W. Kim[1], Susan F. Rudy[6], Vishnu R. Kannabiran[6], Xinping Yang[6], Minyoung Jang[6], Zhong Chen[6], Nanette Suksta[6], Theresa Cooley-Zgela[4], Susmita G. Ramanand[7], Aarif Ahsan[7], Mukesh K. Nyati[7], John J. Wright[5], and Carter Van Waes[4,6]

Abstract

Purpose: A phase I clinical trial and molecular correlative studies were conducted to evaluate preclinical evidence for combinatorial activity of the proteasome inhibitor bortezomib, the epidermal growth factor receptor (EGFR) inhibitor cetuximab, and radiation therapy.

Experimental Design: Patients with radiotherapy-naive stage IV or recurrent squamous cell carcinoma of the head and neck (SCCHN) were studied. Escalating doses of bortezomib (0.7, 1.0, and 1.3 mg/m^2) were given intravenously twice weekly on days 1, 4, 8, and 11, every 21 days, with weekly cetuximab beginning 1 week prior and concurrently with intensity-modulated radiotherapy, delivered in 2 Gy fractions to 70 to 74 Gy. Molecular effects were examined in serial serum and SCCHN tumor specimens and the cell line UMSCC-1.

Results: Seven patients were accrued before the study was terminated when five of six previously untreated patients with favorable prognosis oropharyngeal SCCHN progressed within 1 year (progression-free survival = 4.8 months; 95% CI, 2.6–6.9). Three patients each received bortezomib 0.7 or 1.0 mg/m^2, without dose-limiting toxicities; one patient treated at 1.3 mg/m^2 was taken off study due to recurring cetuximab infusion reaction and progressive disease (PD). Expected grade 3 toxicities included radiation mucositis ($n = 4$), dermatitis ($n = 4$), and rash ($n = 1$). SCCHN-related cytokines increased in serial serum specimens of patients developing PD ($P = 0.029$). Bortezomib antagonized cetuximab- and radiation-induced cytotoxicity, degradation of EGFR, and enhanced prosurvival signal pathway activation in SCCHN tumor biopsies and UMSCC-1.

Conclusions: Combining bortezomib with cetuximab and radiation therapy showed unexpected early progression, evidence for EGFR stabilization, increased prosurvival signaling, and SCCHN cytokine expression, warranting avoidance of this combination. *Clin Cancer Res; 17(17); 5755–64. ©2011 AACR.*

Authors' Affiliations: [1]Hematology-Oncology and Head and Neck Cancer Program, and [2]Radiation Oncology, University of Pittsburgh, Pittsburgh, PA; [3]Early Clinical Trials Development, Division of Cancer Treatment and Diagnosis, [4]Radiation Oncology Branch, and [5]Cancer Therapy Evaluation Program, National Cancer Institute; [6]Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, Maryland; and [7]Department of Radiation Oncology, University of Michigan, Ann Arbor, Michigan

Corresponding Author: Carter Van Waes, National Institute on Deafness and Other Communication Disorders, NIH, Building 10, CRC, Room 4-2732, Bethesda, MD 20892. Phone: 301-402-4216; Fax: 301-402-1140; E-mail: vanwaesc@nidcd.nih.gov or Athanassios Argiris, Division of Hematology-Oncology, University of Pittsburgh, 5150 Centre Avenue, UPMC Cancer Pavilion, 5th Floor, Pittsburgh, PA 15232. Phone: 412-648-6507; Fax: 412-648-6579; E-mail: argirisae@upmc.edu

Introduction

Epidermal growth factor receptor (EGFR) is upregulated in many cancers, including approximately 90% of squamous cell carcinomas of the head and neck (SCCHN), in which it is associated with decreased patient survival (1, 2). Cetuximab (ERBITUX) is a humanized chimera of C225 that is Food and Drug Administration–approved for use in combination with radiation for the treatment of SCCHN. A phase III clinical trial showed that the addition of cetuximab to radiotherapy results in an approximately 10% improvement in survival over radiotherapy alone in patients with locally advanced SCCHN, particularly those of the oropharynx (3). EGFR is implicated in cellular transformation, cell-cycle progression, DNA repair, prosurvival signal pathway activation, and angiogenesis (4–8). Inhibition of EGFR by anti-EGFR monoclonal antibody C225 has been shown to block

American Association for Cancer Research

Translational Relevance

Epidermal growth factor receptor (EGFR) inhibitor cetuximab and radiotherapy are approved for squamous cell carcinoma of the head and neck (SCCHN), but the added benefit is limited to a subset of patients. EGFR inhibitors attenuate signaling via mitogen-activated protein kinases and STAT3, whereas proteasome inhibitors block activation of nuclear factor-kappa B, another signal-activated transcription factor important in survival of SCCHN. Combined treatment with proteasome and EGFR inhibitors, or these agents individually with radiation, showed cytotoxic activity in preclinical and/or clinical studies. In this phase I trial, combining bortezomib with cetuximab and radiation therapy showed unexpectedly short progression-free survival that led to early study termination. There was evidence that bortezomib antagonized cetuximab- and radiation-induced degradation of EGFR and enhanced prosurvival signal pathway activation and cell survival. Further clinical studies of proteasome inhibitors in combination with other therapies in SCCHN should be undertaken with caution.

pathways leading to inhibition of tumorigenesis and sensitization of EGFR-driven tumors. Resistance of remaining SCCHN to EGFR inhibitors has been attributed to EGFR overexpression, mutations, or EGFR-independent mechanisms that coactivate multiple signal pathways important for cancer cell survival (1, 2, 9–13).

Several prosurvival pathways have been reported to be variably activated by EGFR and other signals in SCCHN, including the mitogen-activated protein kinases (MAPK), AKT, nuclear factor-kappa B (NF-κB), and STAT3 pathways (8–11). Among these, studies using SCCHN cell lines have revealed that aberrant signaling by cytokine and other growth factor pathways mediate EGFR-independent activation of NF-κB (9, 12). NF-κB is a key family of signal-activated transcription factors that affect prosurvival gene activation, the malignant phenotype, and prognosis (12). Bortezomib (VELCADE, PS-341) is an inhibitor of the 26S proteasome, a macromolecular complex important in degradation of proteins, including inhibitors of kappa B (IκB), that can block activation of NF-κBs (14). In preclinical and phase I studies, bortezomib was shown to inhibit NF-κB activation and has cytotoxic, antiangiogenic, and radiosensitizing activity in SCCHN and other tumors (15–18). However, in combination with reirradiation, bortezomib showed limited clinical activity and lacked the ability to inhibit activated components of the EGFR-inducible MAPK and STAT3 pathways (18, 19). Together, preclinical and clinical results suggested that EGFR inhibitor–dependent signal pathways and NF-κB proteasome–dependent pathways are independently activated and contribute to the malignant phenotype and clinical response of SCCHN (8, 9, 19). Combined treatment with either of these agents individually with radiation, or with

proteasome and EGFR inhibitors, had cytotoxic activity in preclinical and/or early-phase clinical studies (1, 2, 16, 17, 20–24).

We conducted a phase I study to examine the effects of combination of bortezomib–proteasome and cetuximab–EGFR inhibition with intensity-modulated radiation therapy (IMRT) in patients with advanced SCCHN. The primary objectives included evaluation for the toxicities and the maximum tolerated dose (MTD) of this combination. Secondary objectives included clinical response, progression-free survival (PFS), and overall survival (OS). Correlative studies evaluated the effects of combined bortezomib and cetuximab to inhibit activation of the EGFR, MAPK, AKT, STAT3, and NF-κB signal pathways, tumor cell survival, and levels of proinflammatory and angiogenic cytokines regulated by these pathways and detectable in serum of patients with SCCHN.

Materials and Methods

Patient selection

Protocol NCI-7893 was conducted at the NIH and the University of Pittsburgh after obtaining approval by the respective Institutional Review Boards and informed consent. Eligibility criteria included age 18 years or older; pathologically confirmed SCCHN or poorly/undifferentiated carcinoma of any head/neck site except the nasopharynx; previously untreated stage IV disease, residual disease or regional recurrence, without or with distant metastatic disease at less than 3 cm; Eastern Cooperative Oncology Group (ECOG) performance status 0 to 1; adequate organ function; recovery from any prior surgery or chemotherapy including prior cisplatin for more than 3 months; and no prior systemic EGFR inhibitors, bortezomib, head and neck radiation, uncontrolled intercurrent illness; or grade 2 or more peripheral sensory neuropathy.

Treatment plan and patient assessments

The schema for the treatment plan and correlative studies is shown in Figure 1. A standard 3 + 3 dose escalation design [3 subjects without, or up to 6 subjects after a dose limiting toxicity (DLT) per dose level] was planned. Bortezomib (0.7, 1.0, and 1.3 mg/m^2) was given intravenously twice weekly on days 1, 4, 8, and 11, every 21 days. To obtain serum and optional tumor biopsies with the drug combination without and with radiation, bortezomib and cetuximab 400 mg/m^2 were started 1 week before combining bortezomib and weekly cetuximab 250 mg/m^2 with IMRT. Tumor received 2 Gy per fraction once daily 5 days per week to 70 to 74 Gy. Regions of intermediate and low risk received 60 to 64 and 50 Gy, respectively. Bortezomib (Millennium Pharmaceuticals Inc.) was provided through a Clinical Trials Agreement, Cancer Therapeutics Evaluation Program, National Cancer Institute (NCI).

Baseline evaluation included history, physical examination, standard laboratory tests, and computed tomographic (CT) or CT-positron emission tomographic (PET) imaging
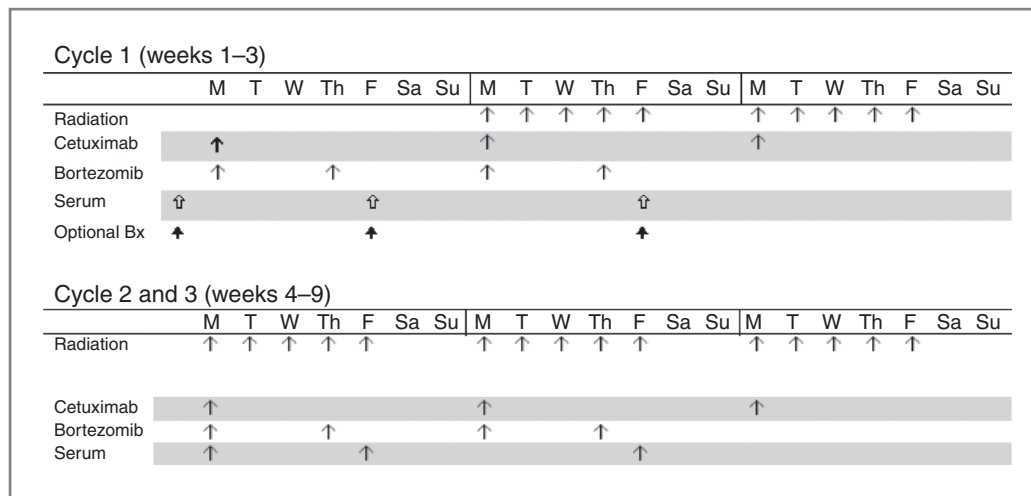
Figure 1. Dosing schema NCI-7893 for bortezomib with weekly cetuximab and IMRT. Patients were given escalating doses of bortezomib (0.7, 1.0, and 1.3 mg/m²), twice weekly by intravenous administration on days 1, 4, 8, and 11 every 3 weeks. Bortezomib and cetuximab (400 mg/m² loading dose, bold arrow) were started during week 1, followed by bortezomib and weekly cetuximab (250 mg/m², nonbold arrows) concurrent with IMRT 2 Gy/d, 5 d/wk, to 70 to 74 Gy. Serum was collected as indicated for SCCHN-related cytokines and optional tumor biopsies were obtained before and during the first cycle of treatment as indicated.

of the head, neck, and chest obtained within 2 weeks of treatment. During treatment, patients underwent weekly physical examination, toxicity evaluation, complete blood cell count, and blood chemistries. Toxicities were assessed by NCI Common Terminology Criteria for Adverse Events (CTCAE; available from: http://ctep.cancer.gov/protocol-development/electronic_applications/docs/ctcaev3.pdf). Collection of serum was planned before and during each cycle, and optional tumor biopsies were planned pretreatment and weeks 1 and 2 of cycle 1 as in Figure 1. Tumor measurements were carried out at baseline and 2 and 5 months after completion of radiotherapy. Response and progression were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST; ref. 25).

Study endpoints

The primary endpoints included DLTs, other toxicities, and the MTD of bortezomib for this combination regimen. Patients were evaluable for toxicity if they received one cycle of therapy or if they had DLT during the first cycle. Evaluation for DLT included the period on drug/radiation treatment plus 4 weeks of follow-up. DLTs were defined as CTCAE 3.0 grade 4 toxicities for the following: in-field stomatitis/mucositis, dermatitis or dysphagia lasting more than 5 days; rash; nausea/vomiting despite appropriate antiemetic therapy; absolute neutropenia of less than 500/μL for more than 7 days, or neutropenic fever; thrombocytopenia; and recurrent grade 4 hematologic toxicities following delay or dose modification. Other DLTs included grade 3 or recurrent grade 2 neuropathy despite dose delay or modifications; all other grade 3 or higher toxicities, except grade 3 fatigue; infection without grade 4 neutropenia; in-field toxicities and nausea/vomiting as discussed previously; weight loss; dehy-

dration; creatinine; hypotension; anorexia; pain; and any grade hypomagnesemia, hypokalemia, or hyponatremia. DLT also included treatment delay due to toxicity of more than 3 weeks, except cetuximab infusion reactions of grade 3 or more, for which study removal and replacement were planned. Toxicities attributable (possible, probable, or definite) to the study treatment were used for determination of DLT and MTD.

Secondary clinical endpoints included objective response, PFS, and OS. Secondary correlative endpoints included pre- to posttreatment changes in a set of serum and tumor biomarkers.

Serum cytokine and growth factor assays

Concentrations of serum cytokine and growth factors were determined as described previously (18, 26). Peripheral blood sample collection was planned within 2 weeks of the initiation of treatment and then after the initiation of study drugs on days 1, 5, and 12 of the first cycle. Thereafter, optional blood for serum could be collected on days 1, 5, and 12 of the second and third cycles (weeks 5 and 8) of bortezomib; following completion of radiotherapy at 8 weeks; and up to 3, 6, 12, 15, 18, 21, and 24 months. See Supplementary Methods for details.

Correlative studies of SCCHN tumor and cell line UMSCC-1

Serial SCCHN tumor biopsies were obtained pretreatment, on day 5 after induction with bortezomib and cetuximab, and on day 12 after combination with IMRT from one patient (#7) who consented to optional biopsies. To confirm and elucidate the mechanism of results obtained, HNSCC cell line UM-SCC1 was treated with bortezomib, cetuximab, and/or radiation. Methods for

Table 1. Patient and tumor characteristics

Patient number	Age, y	Gender	Stage/resectability	Primary site	HPV and p16 status	Smoking history	Alcohol history
1	52	Male	Original T×N2aM0, recurrence tonsil, neck resectable	Tonsil	N/A	Former 35 pk/y	No
2	48	Male	T3N2cM0 resectable	Base of tongue	HPV+	Current 68 pk/y	No
3	58	Male	T1N2aM0 resectable	Base of tongue	HPV+	Never	Yes
4	62	Male	T4N2cM0 resectable	Base of tongue	HPV−/p16+	Former weekly pipe/cigar	Yes
5	61	Male	T3N2cM0 unresectable	Base of tongue	HPV+	Former 48 pk/y	Yes
6	50	Female	T3N2cM0 resectable	Larynx	HPV−	Former 35 pk/y	Yes
7	54	Female	T1N2AM0 resectable	Tonsil	HPV+/p16+	Current 30 pk/y	Yes

NOTE: Patient 1 presented with incomplete TxN2aM0 staging and treatment consisting of neck dissection only, after which he sought care with recurrent tonsil and neck disease.

Abbreviations: TNM, tumor, node, metastasis; pk/y = cigarette pack-years; N/A, not available.

immunoblotting of SCCHN tumor and UMSCC-1 (13, 17) and clonogenic survival assays (17) for correlative studies were described previously and as modified in Supplementary Methods.

Statistics

Using the standard $3 + 3$ design, dose escalation is based on a 33% or less true rate of DLT in 3 patients, and MTD on a 16% or less true rate if the cohort is expanded to 6 subjects for a DLT (18). On the basis of previous studies linking increasing cytokine levels with progressive disease (PD; ref. 26), an exploratory comparison of the PFS between patients whose early cytokine changes after initiating treatment tended to increase and those whose values tended to decrease or remain steady was done using an exact log–rank test. For clonogenic survival assays, the difference in the surviving fraction after combination of drug treatments and 2 Gy irradiation was compared, as the surviving fraction in cell lines has been reported to correlate with the radio-curability of the corresponding human tumors *in vivo* (27).

Results

Patient characteristics, treatment, and response

Subjects (Table 1) included 6 previously untreated patients with stage IV disease and 1 patient who presented after incomplete staging and surgical neck dissection with recurrent tonsil and neck SCCHN; 6 had oropharyngeal and 1 had a laryngeal primary. Treatment delivery (Supplemental Table S1) was completed in the first 6 patients, although bortezomib dose reduction was required in one dose level 2 patient for thrombocytopenia. Patient 7 (1.3 mg/m² dose level) was taken off study after 8 doses of bortezomib and 6 doses of cetuximab for recurrent grade 2 cetuximab infusion reactions and PD. There were no DLTs, and an MTD for combination of bortezomib with cetuximab and radiation was not reached before the study was ended. Clinical outcomes (Table 2) precipitated termination of the study after 5 of 6 of the previously untreated patients exhibited progression within 1 year. Only 3 of 7 patients achieved a complete response (CR) within 2 to

Table 2. Treatment and outcomes

Patient number	Bortezomib dose level, m⁻²	Best objective response	Disease progression	PFS, mo	Site of progression	Salvage therapy	OS, mo	Survival status
1	0.7 mg/m²	CR	No	14	None	None	14	NED
2	0.7 mg/m²	PD	Yes	6	Distant	None	8	DOD
3	0.7 mg/m²	PD	Yes	5	Regional	Neck surgery	17	NED
4	1 mg/m²	CR	Yes	11	Distant	Lobectomy	23	NED
5	1 mg/m²	PD	Yes	5	Local	None	17	DOD
6	1 mg/m²	CR	No	24	None	None	24	NED
7	1.3 mg/m²	PD	Yes	1	Regional	Chemoradiotherapy	18	NED

NOTE: Best response by CT-PET at 2 or 5 months posttreatment. Clinical progression-free and overall survival as of last visit or December 1, 2010.

Abbreviations: NED, no evidence of disease; DOD, died of disease.

5 months (patients 1, 4, 6). Of these, patient 4 developed a solitary pulmonary metastasis at 11 months. Three patients had PD during (patient 7) or within 5 months of treatment (patients 3 and 5). Overall, 3 had locoregional and 2 had pulmonary PD. Locoregional failures occurred within the radiation treatment region.

The median PFS was only 4.8 months (95% CI, 2.6–6.9), including early recurrence in five of 6 patients with previously untreated oropharynx cancer, which compared unfavorably with a median PFS of 17 months reported for cetuximab and radiation at that site (3). Although stratification for tumor site and testing for human papillomavirus (HPV) or p16 status were not incorporated in design of this phase I study, of 6 patients with oropharyngeal primary site lesions, patients 2, 3, and 5 were reportedly HPV+ prior to study entry, and specimens from PD lesions in patients 4 (lung) and 5 (oropharynx) tested p16+ at the site of PD, consistent with HPV origin. Of the 5 with PD, patients 2, 4, 5, and 7 had other cofactors considered to increase risk of recurrence (T stage ≥ 3 or unresectable; N stage ≥ 2; and current or former smoking ≥ 10 pack/y). Early detection of PD and salvage therapy by parotidectomy (patient 3), lobectomy (patient 5), or cisplatin concurrent with remaining radiation (patient 7) achieved disease-free status in 3 of 5 recurrent patients, who together have a median OS of 18 months at last follow-up. The patient with a laryngeal primary and CR remains disease-free after 24 months.

Toxicities

Toxicities (Table 3) included expected grade 3 toxicities for the treatment combination, such as mucositis ($n = 4$), dysphagia ($n = 3$), xerostomia ($n = 1$), and dermatitis ($n = 1$); cetuximab-associated acneiform rash ($n = 1$); and bortezomib-associated peripheral neuropathy ($n = 1$). One grade 3 infection occurred in a patient without neutropenia.

Correlative studies of serum cytokines

Previously, a pretreatment increase in multiple tumor-related cytokine and angiogenic growth factors was detected in patients with SCCHN (26). On the basis of the rationale that this set of cytokines was coregulated by NF-κB, the predictive value of coordinate changes in 3 or more of these cytokines was evaluated. Longitudinal increases in 3 or more of these factors were associated with decreased response and survival in patients with oropharyngeal SCCHN. Consistent with previous findings, increases in 3 or more cytokines occurred in 4 patients (2, 3, 5, and 7) who developed PD and increases in 2 or fewer cytokines, or decline, in 3 patients with CRs (1, 4, 6; Fig. 2). Although based on fewer patients, there was evidence suggesting that those patients whose initial cytokine profile was generally associated with increasing values after starting treatment were more likely to have shorter PFS than those whose cytokine levels tended to decline with greater PFS ($P = 0.029$ by exact 2-tailed log-rank test). The patient showing the greatest increase in all 4 cytokines during cycle 1 (#7) was treated with the highest dose of bortezomib (1.3 mg/m^2) and developed PD in the neck by week 5 while on treatment.

Correlative studies of markers of prosurvival signal, transcription factors, and apoptosis

We examined the pharmacodynamic effects of bortezomib and cetuximab on EGFR, downstream signal, and apoptosis markers we previously validated for SCCHN in multiple studies (8, 10, 11, 15, 17–20). Only patient 7 consented to optional serial biopsies of SCCHN primary tumor, which were obtained pretreatment, on day 5 after induction with bortezomib 1.3 mg/m^2 (days 1 and 4) and cetuximab 400 mg/m^2 (day 1), and on day 12, after combination with IMRT. Figure 3A shows that by day 5, combination of bortezomib and cetuximab enhanced, rather than inhibited, phosphorylated and total EGFR, pERK1/2, and NF-κB p65 subunit. By day 12, with the addition of IMRT, further enhancement of phosphorylated and total EGFR, pAKT, STAT3, and NF-κB p65 was observed. Increase in cleaved PARP as an indicator of cytotoxicity was detected only after the initiation of IMRT (day 12).

Molecular effects in SCCHN in vitro

To further determine how these effects observed in tumor specimens were related to the activity of the individual or combination of agents, SCCHN cell line UMSCC-1 was treated as indicated and effects were examined by clonogenic survival assay and Western blot analysis for EGFR and downstream signaling components (Fig. 3B and C). Combination of C225 or bortezomib and radiation reduced clonogenic survival (Fig. 3B). However, combination of cetuximab and bortezomib when combined with radiation reduced the overall effect of treatment to a level intermediate between that observed with either C225 or bortezomib with radiation and the control (Fig. 3B). Reduction of survival was accompanied by reduction in EGFR and pEGFR (Fig. 3C). Inhibition of one or more downstream signal mediators including pAKT, pERK, and pSTAT3 was often observed with C225, or combination of C225 and radiation, but bortezomib attenuated these effects (Fig. 3C). These findings may explain the reduced efficacy of C225 and radiation when combined with bortezomib, which can inhibit proteasome activity, and possibly, C225-induced EGFR degradation.

Discussion

The combination of bortezomib, cetuximab, and IMRT was tolerated with supportive care but resulted in a median PFS of only 4.8 months. These poor efficacy results included 5 of 6 previously untreated patients with HPV- and/or p16-positive oropharyngeal carcinomas, which compared unfavorably with results of 17.1 months for oropharynx site tumors reported for cetuximab and radiotherapy (3). This group has been associated with favorable prognosis in additional studies, even though unfavorable

Table 3. Worst toxicities (*n* = 7)

|	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	6	1	0	0	0
Anemia	3	3	1	0	0
Thrombocytopenia	5	0	2	0	0
Lymphopenia	4	0	0	1	2
Infection with normal or grade 1/2 neutrophils	5	0	0	2	0
Infection, other	5	0	2	0	0
Mucositis/stomatisis (clinical examination)	0	0	3	4	0
Dysphagia	0	0	3	4	0
Radiation dermatitis	0	1	2	4	0
Rash–-acneiform	4	0	2	1	0
Rash—desquamation	5	1	1	0	0
Dermatology—other	5	1	1	0	0
Allergic reaction/hypersensitivity (drug fever)	6	0	1	0	0
Motor and sensory neuropathy	5	0	1	1	0
Muscle weakness, generalized, whole body	6	1	0	0	0
Diarrhea	5	2	0	0	0
Constipation	4	1	2	0	0
Anorexia	4	1	2	0	0
Nausea	2	3	2	0	0
Vomiting	3	3	1	0	0
Weight loss	4	1	2	0	0
Elevated alkaline phosphatase	5	2	0	0	0
Elevated liver transaminases, aspartate aminotransferase	5	2	0	0	0
Elevated liver transaminases, alanine aminotransferase	2	5	0	0	0
Fever without neutropenia	4	3	0	0	0
Gastrointestinal–-other	4	1	2	0	0
Hiccoughs	6	1	0	0	0
Allergic rhinitis	5	1	1	0	0
Fatigue/asthenia	0	1	6	0	0
Cytokine release syndrome	6	0	1	0	0
Diaphoresis	6	1	0	0	0
Dysarthria/voice changes	6	0	1	0	0
Dysgeusia/taste changes	4	1	2	0	0
Dyspepsia	6	0	1	0	0
Edema	6	1	0	0	0
Hyperbilirubinemia	6	1	0	0	0
Hypermagnesemia	4	3	0	0	0
Potassium, serum-high	6	1	0	0	0
Hypoalbuminemia	4	1	2	0	0
Hypocalcemia	5	1	1	0	0
Hypomagnesemia	5	1	1	0	0
Hyponatremia	4	3	0	0	0
Hypophosphatemia	5	1	0	1	0
Hypotension	5	0	2	0	0
Insomnia	6	1	0	0	0
Pain, all types	0	0	4	3	0
Rigors, chills	5	1	1	0	0
Sinonasal reactions	4	2	1	0	0
Skin breakdown/decubitus ulcer	6	0	1	0	0
Xerosis/dry skin	3	3	1	0	0
Xerostomia/dry mouth	3	3	1	0	0
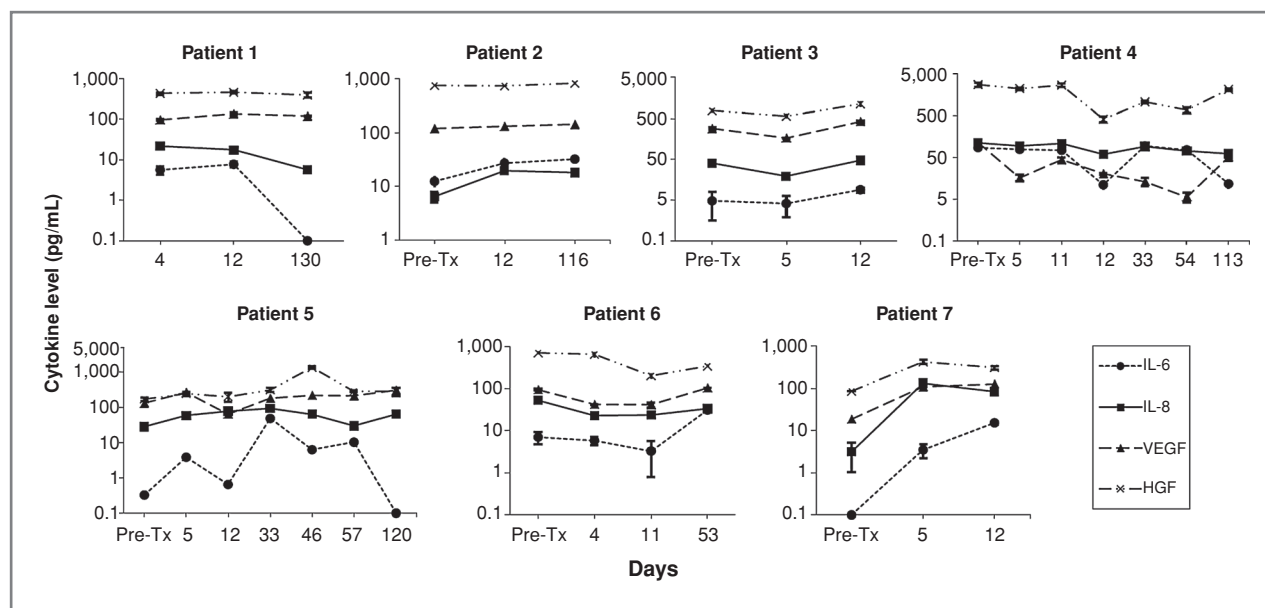
Figure 2. Longitudinal changes in serum cytokine levels in 7 patients. Based on the rationale of coregulation of 5 serum cytokines by NF-κB, the predictive value of coordinate changes in 3 or more of these cytokines was evaluated, as previously (23). Cytokine concentrations (pg/mL) are presented as mean ± SD of replicates on a log scale versus days since beginning treatment. Pre-Tx, pretreatment. Longitudinal increase is slope of 3 or more cytokines previously associated with poor response and survival in patients with oropharyngeal carcinoma (23) was seen in patients 2, 3, 5, and 7 and associated with decreased PFS ($P = 0.029$, log-rank test). IL, interleukin; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor.

characteristics (advanced stage and history of heavy smoking) that can influence outcome were also present (28). Although it is possible that these adverse risk factors contributed to the unexpectedly low response and early recurrence in the small cohort in the present study, translational studies provided additional evidence for an adverse interaction of the combination of bortezomib, cetuximab, and radiation. Greater than expected EGFR and cell survival signaling, and angiogenesis factor expression by SCCHN, was observed. Together, the clinical and molecular findings caution against further clinical investigation of this combination of agents.

The clinical results of this study were initially surprising after early preclinical and clinical studies provided evidence that combined treatment with either of these agents individually with radiation, or a combination of proteasome and EGFR inhibitors, potentiated cytotoxic activity (1, 2, 16, 17, 20–24). However, evidence emerging from one of our laboratories concurrent with this trial indicated that proteasome inhibitors could potentially antagonize chemotherapy or radiation-induced EGFR degradation and antiproliferative and cytotoxic effects (M. Nyati, unpublished observations; ref. 29). Consistent with this possibility, analysis of serial tumor biopsies from the patient who developed PD on-treatment revealed increased EGFR and prosurvival signaling instead of EGFR degradation and attenuation of prosurvival signaling previously reported with cetuximab or cetuximab and radiation (29). Further studies in the UMSCC-1 cell line showed that combination of C225 or bortezomib with radiation reduced clonogenic

survival consistent with previous preclinical studies (Fig. 3B), but combination of cetuximab and bortezomib with radiation reduced the overall effect of treatment to a level intermediate between that observed with either drug used with radiation and the control (Fig. 3B). Bortezomib also attenuated the effects of cetuximab- and radiation-induced EGFR degradation and inhibition of prosurvival signaling in UMSCC-1 (Fig. 3C).

Because recent evidence suggests that EGFR is degraded by the ubiqutin-proteasome system (30, 31), it seems likely that proteasome inhibition by bortezomib could attenuate the cytotoxic effects of cetuximab and radiation by protecting EGFR from degradation. Furthermore, recent reports show that proteasome inhibitor–induced activation of EGFR and EGFR-independent mechanisms can induce MAPK, AKT, and STAT3 prosurvival pathways, as observed here (9, 19, 32–34). In addition, whereas proteasome inhibitors radiosensitized cancer cells and smaller xenograft tumors in experimental models (15–17), they may enhance radioprotection of SCC tumor cells under hypoxic conditions (35), such as occur in large SCCHN in advanced stage patients. Cytokines and angiogenesis factors expressed by SCCHN in response to prosurvival (26) and hypoxia signals (36) were detected in serum of all 7 patients pretreatment. Concentrations of 3 or more serum cytokine and angiogenic growth factors previously shown to increase with poor response and survival in patients with oropharyngeal SCCHN (26) increased in the 4 patients with early PD. Others and we have shown that these cytokines may be produced in patient tumors
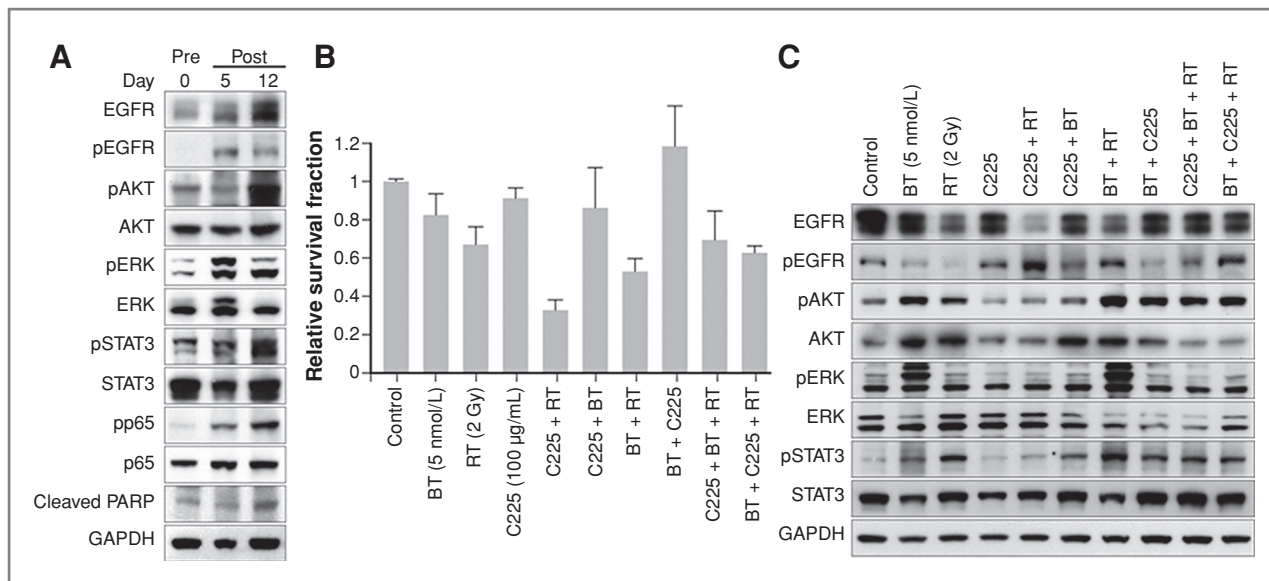
Figure 3. Combined bortezomib (BT), cetuximab, and radiotherapy (RT) enhances coactivation of EGFR and multiple prosurvival pathways in SCCHN tumor biopsies, along with clonogenic survival in line UMSCC-1. A, tumor biopsies were obtained from patient 7 before and on day 5 after initiating combined treatment with bortezomib and cetuximab and on day 12 after the addition of IMRT. Protein extracts from tumor specimens were subjected to SDS-PAGE, and Western blot analyses were carried out for activated EGFR and signal phospho- and total proteins shown. Combined bortezomib and cetuximab treatment increased phosphorylation and total EGFR and phosphorylation of downstream prosurvival signal kinases and transcription factors, including pAKT, pERK1/2, pSTAT3, and pNF-κB p65. B, clonogenic survival assays. UMSCC-1 cells were treated with bortezomib, cetuximab, and radiotherapy alone or in combinations as indicated. Clonogenic assays were carried out, and surviving fractions are presented. C, Western blot analyses were carried out for activated EGFR and signal phospho- and total proteins shown. Combined bortezomib and cetuximab treatment with radiotherapy resulted in stabilization of EGFR and phosphorylated EGFR and downstream prosurvival signal kinases and transcription factors, including pAKT, pERK1/2, and pSTAT3. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

and cell lines by SCCHN epithelial and stromal cells (37, 38). We have further shown that cytokines such as interleukin 8 (IL-8) may be induced in SCCHN lines by bortezomib through activation of MAPK signaling and transcription factor activator protein 1 (AP-1; ref. 32). Thus, proteasome inhibitor- and EGFR-induced expression of IL-8, VEGF, and HGF could enhance angiogenesis, tumorigenesis, and metastasis (32, 37, 38).

Together, the stabilization or enhancement of EGFR-mediated survival signaling and angiogenesis factor expression may help to further explain the suboptimal efficacy of the combination of these drugs with radiation. These observations suggest that cetuximab and radiation have multiple effects on cancer besides DNA repair and that combination studies should be pursued with caution. Both drug–drug and drug–radiation interactions affecting diverse mechanisms may need to be considered when developing therapeutic regimens.

The first in-human phase I study of bortezomib in combination with reirradiation for recurrent SCCHN was also recently concluded at NIH. Although bortezomib inhibited proteasome, NF-κB p65 subunit, and prosurvival genes (18), clinical activity of bortezomib plus reirradiation was limited (19). PRs were seen in 5 of 10 patients receiving lower doses and bortezomib treatment breaks, whereas PD occurred in patients receiving a continuous schedule or higher doses of bortezomib with reirradiation. The limited clinical activity observed was also associated

with lack of inhibition of EGFR-activated extracellular signal-regulated kinase (ERK) or STAT3 pathways, as well as other noncanonical NF-κB/REL family members, which may also contribute to cell survival (19). A recent phase I study of bortezomib plus cetuximab in treatment-refractory patients with tumors expressing EGFR yielded stable disease (SD) but no PRs or CRs in 5 of 6 patients with SCCHN or lung cancer (24). Altogether, the results of these studies show that bortezomib in combination with cetuximab or reirradiation results in incomplete clinical and molecular responses in SCCHN.

Recently completed phase II studies of bortezomib with other chemotherapies for recurrent SCCHN also showed evidence of limited combinatorial activity or possible chemoprotection (39, 40). One of these studies showed that the response rate was lower than expected for docetaxel alone and PD was associated with an increased NF-κB and EGFR gene profile (39). Another phase I trial evaluated bortezomib in combination with weekly cisplatin 30 mg/m^2 and radiotherapy for advanced SCCHN (41). Twenty-seven patients with previously untreated locoregionally advanced (10 patients) or recurrent/previously irradiated (17 patients) SCCHN were studied. Only 8 patients (30%) were without PD at a median 7.3-month follow-up. Interestingly, there is now evidence that proteasome inhibition may antagonize chemotherapy-mediated EGFR degradation and cytotoxicity as well. Gemcitabine or cisplatin chemotherapy cytotoxicity was shown to involve

ubiquitination and proteasome-dependent EGFR degradation (30, 42).

In conclusion, the present and other clinical and mechanistic studies suggest that bortezomib may have limited clinical efficacy, and in some instances, lower than expected activity due to antagonism, when combined with cetuximab and other cytotoxic therapies of known activity in SCCHN. Proteasome inhibitor–mediated activation of EGFR-dependent and independent MAPK, AKT, or STAT3 prosurvival signaling may be countered by combination with ERK, c-*jun*, kinase (JNK), and AKT inhibitors (31, 32). However, as learned here and other recent trials cited earlier, further study of proteasome inhibitors in combination with other targeted therapies should be considered only with caution after testing in appropriate non-HPV and HPV+ HNSCC xenograft models appropriate for the patient population to be studied. These results also provide several insights important in avoiding or reducing the impact of unfavorable outcomes in the future. Despite the inherent challenges and limitations in preclinical modeling of the combination and sequencing of multiple therapies to be used in clinical trials, accurate modeling is important to identify potential interactions and mechanisms that could result in unfavorable clinical outcomes. Close monitoring is important for early recognition of unfavorable outcomes for provision of additional therapy, early stoppage of the study, and reporting. Obtaining paired pre- and on-treatment specimens for correlative studies can support the identification of possible underlying mechanisms.

References

1. Rubin Grandis J, Melhem MF, Gooding WE, Day R, Holst VA, Wagener MM, et al. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 1998;90:824–32.
2. Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res 2002;62:7350–6.
3. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567–78.
4. Huang SM, Harari PM. Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. Clin Cancer Res 2000;6:2166–74.
5. Harari PM, Huang SM. Head and neck cancer as a clinical model for molecular targeting of therapy: combining EGFR blockade with radiation. Int J Rad Oncol Biol Phys 2001;49:427–33.
6. Dittmann K, Mayer C, Rodemann HP. Inhibition of radiation-induced EGFR nuclear import by C225 (Cetuximab) suppresses DNA-PK activity. Radiother Oncol 2005;76:157–61.
7. Kriegs M, Kasten-Pisula U, Rieckmann T, Holst K, Saker J, Dahm-Daphi J, et al. The epidermal growth factor receptor modulates DNA double-strand break repair by regulating non-homologous end-joining. DNA Repair 2000;9:889–97.
8. Bancroft CC, Chen Z, Yeh J, Sunwoo JB, Yeh NT, Jackson S, et al. Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. Int J Cancer 2002;99: 538–48.
9. Lee TL, Yeh J, Van Waes C, Chen Z. Epigenetic modification of SOCS-1 differentially regulates STAT3 activation in response to interleukin-6 receptor and epidermal growth factor receptor signaling through JAK and/or MEK in head and neck squamous cell carcinomas. Mol Cancer Ther 2006;5:8–19
10. Pernas FG, Allen CT, Winters ME, Yan B, Friedman J, Dabir B, et al. Proteomic signatures of epidermal growth factor receptor and survival signal pathways correspond to gefitinib sensitivity in head and neck cancer. Clin Cancer Res 2009;15:2361–72.
11. Van Waes C, Allen CT, Citrin D, Gius D, Colevas AD, Harold NA, et al. Molecular and clinical responses in a pilot study of gefitinib with paclitaxel and radiation in locally advanced head-and-neck cancer. Int J Radiat Oncol Biol Phys 2010;77:447–54.
12. Allen CT, Ricker JL, Chen Z, Van Waes C. Role of activated nuclear factor-kappaB in the pathogenesis and therapy of squamous cell carcinoma of the head and neck. Head Neck 2007;29:959–71.
13. Cohen J, Chen Z, Lu SL, Yang XP, Arun P, Ehsanian R, et al. Attenuated transforming growth factor beta signaling promotes nuclear factor-kappaB activation in head and neck cancer. Cancer Res 2009;69:3415–24.
14. Adams J, Kauffman M. Development of the proteasome inhibitor Velcade (bortezomib). Cancer Invest 2004;22:304–11.
15. Sunwoo JB, Chen Z, Dong G, Yeh N, Crowl Bancroft C, Sausville E, et al. Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-kappa B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. Clin Cancer Res 2001;7:1419–28.
16. Pervan M, Pajonk F, Sun JR, Withers HR, McBride WH. Molecular pathways that modify tumor radiation response. Am J Clin Oncol 2001;24:481–5.
17. Van Waes C, Sunwoo JB, DeGraff W, Mitchell JB. Radiosensitization and proteasome inhibition. In:Adams J, editor. Proteasome inhibitors in cancer therapy. Totowa, NJ: Humana Press; 2004. p. 123–30.
18. Van Waes C, Chang AA, Lebowitz PF, Druzgal CH, Chen Z, Elsayed YA, et al. Inhibition of nuclear factor-kappaB and target genes during combined therapy with proteasome inhibitor bortezomib and reirradiation in patients with recurrent head-and-neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2005;63:1400–12.

19. Allen C, Saigal K, Nottingham L, Arun P, Chen Z, Van Waes C. Bortezomib-induced apoptosis with limited clinical response is accompanied by inhibition of canonical but not alternative nuclear factor-{kappa}B subunits in head and neck cancer. Clin Cancer Res 2008;14:4175–85.

20. Morris JC, Citrin D, Nottingham L, Rudy SF, Harold N, Cooley-Zgela T, et al. Phase I study of proteasome inhibitor bortezomib (B) concurrent with re-irradiation therapy (re-RT) for recurrent squamous cell carcinoma of the head and neck (SCCHN). J Clin Oncol 2010;2815s:abstr 2603.

21. Lorch JH, Thomas TO, Schmoll HJ. Bortezomib inhibits cell-cell adhesion and cell migration and enhances epidermal growth factor receptor inhibitor–induced cell death in squamous cell cancer. Cancer Res 2007;67:727–34.

22. Wagenblast J, Baghi M, Arnoldner C, Bisdas S, Gstöttner W, Ackermann H, et al. Cetuximab enhances the efficacy of bortezomib in squamous cell carcinoma cell lines. J Cancer Res Clin Oncol 2009;135:387–93.

23. Cascone T, Morelli MP, Morgillo F, Kim WY, Rodolico G, Pepe S, et al. Synergistic anti-proliferative and pro-apoptotic activity of combined therapy with bortezomib, a proteasome inhibitor, with anti-epidermal growth factor receptor (EGFR) drugs in human cancer cells. J Cell Physiol 2008;216:698–707.

24. Dudek AZ, Lesniewski-Kmak K, Shehadeh NJ, Pandey ON, Franklin M, Kratzke RA, et al. Phase I study of bortezomib and cetuximab in patients with solid tumours expressing epidermal growth factor receptor. Br J Cancer 2009;100:1379–84.

25. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.

26. Allen C, Duffy S, Teknos T, Islam M, Chen Z, Albert PS, et al. Nuclear factor-kappaB-related serum factors as longitudinal biomarkers of response and survival in advanced oropharyngeal carcinoma. Clin Cancer Res 2007;13:3182–90.

27. Fertil B, Malaise EP. Inherent cellular radiosensitivity as a basic concept for human tumor radiotherapy. Int J Radiat Oncol Biol Phys 1981;7:621–9.

28. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24–35.

29. Feng FY, Lopez CA, Normolle DP, Varambally S, Li X, Chun PY, et al. Effect of epidermal growth factor receptor inhibitor class in the treatment of head and neck cancer with concurrent radiochemotherapy in vivo. Clin Cancer Res 2007;13:2512–8.

30. Feng FY, Varambally S, Tomlins SA, Chun PY, Lopez CA, Li X, et al. Role of epidermal growth factor receptor degradation in gemcitabine-mediated cytotoxicity. Oncogene 2007;26:3431–9.

31. Kesarwala AH, Samrakandi MM, Piwnica-Worms D. Proteasome inhibition blocks ligand-induced dynamic processing and internalization of epidermal growth factor receptor via altered receptor ubiquitination and phosphorylation. Cancer Res 2009;69:976–83.

32. Chen Z, Ricker JL, Malhotra PS, Nottingham L. Differential bortezomib sensitivity in head and neck cancer lines corresponds to proteasome, nuclear factor-kappaB and activator protein-1 related mechanisms. Mol Cancer Ther 2008;7:1949–60.

33. Sloss CM, Wang F, Liu R, Xia L, Houston M, Ljungman D, et al. Proteasome inhibition activates epidermal growth factor receptor (EGFR) and EGFR-independent mitogenic kinase signaling pathways in pancreatic cancer cells. Clin Cancer Res 2008;14:5116–23.

34. Li C, Zang Y, Sen M, Leeman-Neill RJ, Man DS, Grandis JR, et al. Bortezomib up-regulates activated signal transducer and activator of transcription-3 and synergizes with inhibitors of signal transducer and activator of transcription-3 to promote head and neck squamous cell carcinoma cell death. Mol Cancer Ther 2009;8:2211–20.

35. Pajonk F, Grumann T, McBride WH. The proteasome inhibitor MG-132 protects hypoxic SiHa cervical carcinoma cells after cyclic hypoxia/reoxygenation from ionizing radiation. Neoplasia 2006;8:1037–41.

36. Byers LA, Holsinger FC, Kies MS, William WN, El-Naggar AK, Lee JJ, et al. Serum signature of hypoxia-regulated factors is associated with progression after induction therapy in head and neck squamous cell cancer. Mol Cancer Ther 2010;9:1755–63.

37. Chen Z, Malhotra PS, Thomas GR, Ondrey FG, Duffey DC, Smith CW, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. Clin Cancer Res 1999;5:1369–79.

38. Dong G, Chen Z, Li ZY, Yeh NT, Bancroft CC, Van Waes C. Hepatocyte growth factor/scatter factor-induced activation of MEK and PI3K signal pathways contributes to expression of proangiogenic cytokines interleukin-8 and vascular endothelial growth factor in head and neck squamous cell carcinoma. Cancer Res 2001;61:5911–8.

39. Chung CH, Aulino J, Muldowney NJ, Hatakeyama H, Baumann J, Burkey B, et al. Nuclear factor-kappa B pathway and response in a phase II trial of bortezomib and docetaxel in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. Ann Oncol 2010;21:864–70.

40. Gilbert J, Aulino J, Muldowney NJ, Feldman L, Haigentz M, Burtness B, et al. Phase II randomized trial of bortezomib (B) plus irinotecan (I) or B with addition of I at progression in recurrent (R) or metastatic (M) squamous cell carcinoma of the head and neck (SCCHN) (E1304): A trial of the Eastern Cooperative Oncology Group. J Clin Oncol 2009;2715s:abstr 6020.

41. Axelrod RS, Ahn PH, Kubicek GJ, Anne PR, Cognetti D, Dicker A, et al. Mature results of a phase I trial of bortezomib, cisplatin, and radiotherapy for advanced or recurrent head and neck cancer. J Clin Oncol 2010;2815s:abstr 5560.

42. Ahsan A, Hiniker SM, Ramanand SG, Nyati S, Hegde A, Helman A, et al. Role of epidermal growth factor receptor degradation in cisplatin-induced cytotoxicity in head and neck cancer. Cancer Res 2010;70:2862–9.

Clinical Cancer Research

Early Tumor Progression Associated with Enhanced EGFR Signaling with Bortezomib, Cetuximab, and Radiotherapy for Head and Neck Cancer

Athanassios Argiris, Austin G. Duffy, Shivaani Kummar, et al.

Updated version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-11-0861
Supplementary Material	Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2011/07/12/1078-0432.CCR-11-0861.DC1

Cited articles	This article cites 40 articles, 18 of which you can access for free at: http://clincancerres.aacrjournals.org/content/17/17/5755.full#ref-list-1
Citing articles	This article has been cited by 6 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/17/17/5755.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/17/17/5755. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.