A Phase II Study of RT Concurrent with Cetuximab in Patients with Locally Advanced Head and Neck Squamous Cell Carcinoma Who Do Not Qualify For Standard Chemotherapy Due To Age≥70 Or Co-Morbidities (UMCC 2009.009)

Principal Investigator: Shruti Jolly, M.D.[1, 2]
Department of Radiation Oncology

Co-Investigators: Michelle Mierzwa, M.D. [1]
Theodore Lawrence, M.D. Ph.D. [1]

Kemp Cease, M.D.[2]
Susan Urba, M.D.[1]
Francis Worden, M.D.[1]
Carol Bradford, M.D.[1]
Gregory Wolf, M.D.[1]
Mark Prince, M.D.[1, 2]
Jeffrey Moyer, M.D.[1, 2]
Joseph Helman, M.D.[1, 2]
Brent Ward, M.D.[1]
Erin McKean, M.D.[1]
Kelly Malloy, M.D.[1]
Matthew Spector, M.D.[1]
Andrew Shuman, M.D.[1]
Michelle Kim, M.D.[1,2]
Matthew Schipper, Ph.D.[1] (statistician)

[1] University of Michigan Health System
1500 East Medical Center Drive
Ann Arbor, MI 48109

[2] Veterans Affairs Health System
2215 Fuller Road
Ann Arbor, MI 48105

Version: May 3, 2018

TABLE OF CONTENTS

1.0 Introduction

For most patients with locoregionally advanced head and neck cancer, chemoradiotherapy can improve local control and survival, and can achieve organ preservation. This was first demonstrated in the Veterans Affairs Cooperative Study Group prospective randomized landmark trial comparing an organ preservation approach (induction chemotherapy followed by definitive radiation, based on chemotherapy response) to the standard treatment of laryngectomy with radiation [1]. Sixty six percent of the surviving patients had successful larynx preservation with no decrease in survival. Subsequent trials at multiple other institutions have confirmed these results [2-5].

Unfortunately, this improved tumor outcome resulting from the administration of concurrent chemoradiotherapy does not extend to older patients and those with coexisting medical conditions and poor performance status. In the RTOG 91-11 Intergroup Study, the rate of high-grade toxic effects was greater with the chemotherapy-based regimens (81% vs. 61% with radiotherapy alone). The rate of grade 3 and 4 acute mucositis was 20% and 24% in the chemotherapy followed with radiotherapy and the radiotherapy alone arms, respectively, and was doubled in the concurrent chemoradiotherapy arm (43% $p < 0.05$). Also, more deaths that may have been related to therapy were reported in the concurrent chemoradiotherapy arm (5%, compared with 3% in each of the other arms). Enhanced toxicity, primarily mucositis and late dysphagia, were also reported in other studies of concurrent chemoradiotherapy. Mucositis and dysphagia are the main barriers to winning the battle against head and neck cancer [6-9].

Therefore, it is not surprising that age has a dramatic impact on the benefit of concurrent chemotherapy with radiation. A meta-analysis of over 15,000 patients, evaluating the impact of age on patients treated with either concurrent chemoradiotherapy or altered fractionation revealed no benefit to older patients (age>70years)[10]. This lack of benefit is due, in part, to increased morbidity and mortality of treatment. This study addresses the need to develop less toxic therapies for patients who are older and/or have co-morbidities.

EGFR[1] plays a key role in head and neck cancer, in that its over-expression is associated with more aggressive behavior, and its blockade increases the survival of patients treated with radiation without increasing toxicity. We hope to determine the pharmacodynamic profile of response in patients receiving cetuximab and radiation, so that we can determine who benefits from this extremely expensive and moderately toxic therapy.

1.1 Study Hypothesis and Rationale

Bonner *et al* investigated the benefit of the addition of cetuximab to radiation. It was concluded that cetuximab improves locoregional control and reduces mortality without increasing the common toxic effects associated with radiotherapy to the head and neck [11]. In our study, we propose to select locally advanced head and neck cancer patients, who are older than age 70 or are not deemed candidates for chemotherapy. This group was not extensively investigated in the earlier randomized trial, in that

[1] Abbreviations: **CR**, complete response; **EGFR**, epidermal growth factor receptor; **MAPK**, mitogen-activated protein kinase; **PI3K**, phosphatidylinositol 3-kinase; **PR**, partial response; **RT**, radiation treatment/therapy; **STAT**, signal transducer and activator of transcription; **TMA**, tissue microarray.

90% of patients in the Bonner study had a KPS of 80 or greater, and the median age of the patients was 57 [11]. The primary end-point of this trial is to characterize local progression free survival in these patients, when treated with cetuximab+radiation.

We also propose to develop a molecular pharmacodynamic predictor of local control after treatment with combination cetuximab-radiation. We hypothesize that tumor EGFR degradation and other markers of down-stream EGFR inhibition as well as novel phosphoproteomic markers observed in tumor biopsies taken 7 days after the administration of cetuximab, will predict the effectiveness of cetuximab-radiation. The first biopsy will be obtained prior to administration of the loading dose of cetuximab. Prior to initiation of cetuximab concurrent with radiation, on Day 7, following the cetuximab loading dose, a tumor biopsy for the biomarkers will be obtained. We hypothesize that tumors that do not demonstrate the changes that correlate with response after cetuximab in the *in vivo* preclinical studies are more likely to progress following cetuximab-radiation therapy compared to those that show changes that correlate with response. If our hypothesis is validated, we will gain a tool that will help identify patients who are most likely to benefit from radiation and cetuximab, a very expensive and moderately toxic treatment.

In the Bonner trial and in the Vermorken[12] study there was an increased survival benefit that was associated with oropharynx sites. High risk HPV (human papilloma virus) infection has been implicated both as an etiologic factor and as a prognostic factor for survival and response to therapy. HPV is also typically associated with a younger cohort of patients, however as the high risk HPV infected cohort ages, we do expect to see more patients in their 70s and beyond who will have HPV-related head and neck cancers. Thus, we postulate the EGFR inhibition will work better in HPV positive tumors. We will investigate the presence of HPV in the tumor tissue biopsies by a variety of assays. We will also examine the p53 status using RNA isolated from the tumor biopsy since HPV positive tumors most frequently have wild type p53 which may also contribute to the high response rate of these tumors.

1.2 EGFR inhibitors in head and neck cancer

Initial *in vitro* studies demonstrated that prolonged exposure of cells to EGF could enhance the effects of radiation in head and neck cancer cells [13, 14]. It is likely that this radiosensitivity was achieved through EGF-induced EGFR degradation. Additional studies showed that monoclonal antibodies that target EGFR could increase radiation-induced apoptosis[15]. Furthermore, there was an inverse correlation between EGFR expression levels and radiation response [16-18]. This relationship between EGFR expression and poor prognosis was confirmed in human head and neck carcinoma samples [19]. A variety of preclinical studies have demonstrated that EGFR inhibitors can increase radiation sensitivity in both in vitro and in vivo model systems [20-26]. While the majority of studies have reported additive effects resulting from the combination of EGFR antagonists and radiotherapy in vitro, the same combination produces synergistic effects in xenograft models [20, 21, 27-29]. This may be secondary to EGFR inhibitors and radiation impact on several downstream signaling pathways [30]. These include pathways regulating cellular proliferation and apoptosis, which would

be evident in both in vitro and in vivo, and pathways regulating angiogenesis [20, 29] and tumor invasion [31, 32], which might be detectable only in tumor xenograft models.

While preclinical studies have highlighted the potential therapeutic gains that could be achieved by adding EGFR inhibitors to radiation, the best validation of this combination has been from the results of clinical trials in head and neck cancer. A phase III clinical trial demonstrated that, in a cohort of 424 patients with locoregionally advanced squamous cell carcinoma of the head and neck, the addition of cetuximab nearly doubled the median survival of patients (compared to radiotherapy alone), from 28 to 54 months. This study represented the first major success achieved by the addition of an EGFR antagonist to radiotherapy. This improvement was achieved without enhanced toxicity. Notably, the rates of pharyngitis and weight loss were identical in the two arms [11].

1.3 Predicting response to combination cetuximab-radiation

While we hope that we can simply substitute cetuximab for cisplatin-based chemotherapy and achieve the same rate of local control as with chemoradiotherapy without the increased toxicity of chemotherapy, we must also entertain the possibility that only a fraction of these patients will be able to achieve tumor control using this strategy. Therefore, it would be very valuable to be able to predict in advance those patients who will be able to achieve local control with cetuximab and radiation therapy. However, previous efforts to predict the response to EGFR inhibition using the pre-therapy EGFR expression levels have failed. For instance, responses to cetuximab in patients with metastatic colorectal cancer occur in the absence of EGFR staining by immunohistochemistry[33]. Furthermore, the strength of EGFR staining using immunohistochemistry does <u>not</u> predict response to EGFR inhibitors combined with chemotherapy [34]. Previous efforts to predict response in patients with head and neck cancer based on pretreatment EGFR levels have also failed [35]. (Lung cancer may be an exception in this case as mutation of the EGFR may correlate with response [36], but these mutations are rare in head and neck cancer.) One other recent discovery on the biomarkers of response suggests that patients whose tumors do not have K-ras mutations have a significantly higher disease control rate than patients with K-ras mutations when treated with cetuximab in colorectal cancers.[37] However, K-ras mutation in head and neck cancers is rare, therefore this finding will not be useful for these patients. [38]

Although the focus for developing a predictive assay has been on assessing pretreatment paraffin embedded specimens, it seems possible that a predictive assay may require determining the response to treatment (i.e. a pharmacodynamic endpoint). As has been noted by Mendelsohn and Balsega [39], successful application of EGFR targeted therapy may require not only the presence of activated EGFR, but also that the cancer depends on this pathway for cell survival. This dependence ("addiction") might be best determined not simply by assessing pretreatment specimens, but by comparing pre- and post-treatment specimens to determine if the inhibitor has actually inhibited both EGFR phosphorylation and downstream signaling. Indeed, a substantial literature has elucidated these EGFR signaling pathways, which offers candidate molecules. Our preclinical data and the literature

suggest that changes in total EGFR, pEGFR, pSTAT3, and Bcl-X_L, EGFR copy number, EGFRvIII status, and NFκB may predict response to cetuximab-radiation. Additionally, we postulate that EGFR variant 3 (EGFRvIII) expression may predict response to cetuximab, since tumors that express primarily this variant are less likely to respond to cetuximab blockade than tumors that express the full length receptor. The vIII form is reported to be intrinsically active and thus blocking ligand binding with the antibody should not be effective. Sok et al.[40] reported that 42% of head and neck tumors in their series expressed EGFRvIII. We will assess vIII expression using RT-PCR with RNA isolated from the research biopsy.

1.3.1 In addition to the known downstream effectors of EGFR signaling, it is likely that there are additional downstream targets whose status could add to the known predictive markers described above. Proteomic techniques permit one to assess thousands of potential proteins as possible markers. As much of the key signaling is mediated by phosphorylations and dephosphorylations rather than changes in total protein levels, investigators have focused on the phosphoproteome as a likely source of new candidates 41, 42. Several groups have used these approaches to assess EGF stimulation 43, which has confirmed known pathways and elucidated new targets 44. We are unaware of previous efforts to apply this powerful technology toward the discovery of novel predictors of the effectiveness of combined radiation-cetuximab. We are likewise unaware of studies that have tested the clinical response of EGFR variant three expressing head and neck tumors treated with cetuximab. We postulate that tumors expressing only wild type EGFR will be more responsive than those that express variant III.

2.0 Objectives

2.1 Primary

2.1.1 Determine changes in tumor EGFR, pEGFR, downstream signaling, and novel phosphoproteins following a loading dose of cetuximab in patients who are poor candidates for chemoradiation (age ≥70 years or with significant co-morbidities) and are therefore treated with cetuximab and radiation.

2.1.2 Characterize clinical outcomes, including local recurrence, progression-free survival and overall survival in these patients, and correlate these clinical outcomes with the changes in tumor EGFR, pEGFR, downstream signaling, and novel phosphoproteins.

2.1.3 Describe the toxicity, in particular mucositis/dysphagia, of this regimen.

2.2 Secondary

2.2.1 Conduct normal mucosa EGFR assessment for comparison with tumor sample.

2.2.2 Correlate HPV presence and titer with p53 status and clinical outcome.

3.0 Inclusion Criteria

3.1 Patients must have pathologically-confirmed, previously untreated, clinically accessible (without general anesthesia) locally advanced squamous cell carcinoma of the larynx, hypopharynx, oropharynx, oral cavity or nonresectable head and neck squamous cell carcinomas of the skin.

3.2 Patients will be limited to:

- ≥ 70 years of age, **OR**
- with co-morbidities that preclude treatment with standard platinum-based chemotherapy, as determined by the treating physician, **OR**
- KPS≤80, **OR**
- Creatinine clearance <30cc/min

3.3 Laboratory criteria:

3.3.1 WBC ≥ 3500/ul, granulocyte ≥ 1500/ul

3.3.2 Platelet count ≥ 100,000/ul

3.3.3 Total Bilirubin ≤ 1.5 X ULN

3.3.4 AST and ALT ≤ 2.5 X ULN

3.4 Patients must give documented informed consent to participate in this study.

4.0 Exclusion Criteria

4.1 Prior head and neck malignancy, or history of other prior non-head and neck malignancy within the past 3 years (excluding skin cancer and early stage treated prostate cancer).

4.2 Prior head and neck radiation or chemotherapy.

4.3 Documented evidence of distant metastases.

4.4 Patients with nasopharyngeal carcinoma.

4.5 Any medical or psychiatric illness, which, in the opinion of the principal investigator, would compromise the patient's ability to tolerate this treatment.

4.6 Patients with psychiatric/social situations that would limit compliance with study requirements are not eligible.

4.7 Patients with prior anti-epidermal growth-factor receptor antibody therapy (antibody or small molecule).

4.8 Patients residing in prison.

5.0 Pre-treatment

5.1 Complete history and physical examination, examination by Otolaryngology, Radiation Oncology, and Medical Oncology, complete documentation of extent of primary tumor and regional disease.

5.2 Satisfactory biopsy of the primary tumor confirming pathologic diagnosis.

5.3 Complete dental evaluation (at the discretion of physician).

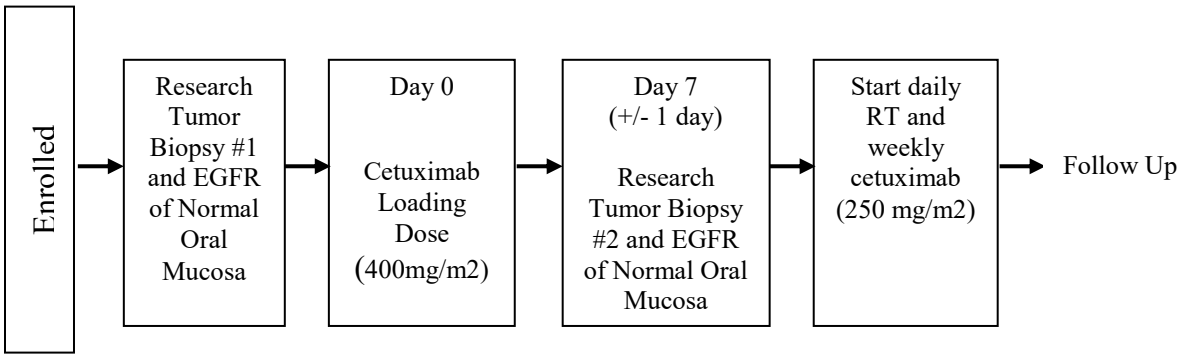
5.4 Completion of laboratory studies: Comprehensive panel, including Magnesium and CBCP with differential.

5.5 Diagnostic CT scan or MRI of the head and neck.

5.6 Initial staging CT-PET scan (at the discretion of physician).

5.7 Baseline toxicity evaluation.

6.0 Schema



7.0 Study Design

7.1 Overview

Patients will receive a single dose of cetuximab 400 mg/m^2 (Day 0). On day 7 (+/- 1 day), a repeat biopsy will be performed. Within approximately 4 days, definitive radiation will begin (70 Gy in 35 fractions to the gross tumor, 50-60 Gy to subclinical target volumes) concurrent with weekly cetuximab 250 mg/m^2. Twelve to 14 weeks following the completion of cetuximab-radiation, patients will undergo standard restaging and treatment, including CT-PET, repeat laryngoscopy and tumor site biopsies. Patients with evidence of persistent tumor may undergo surgical salvage and/or neck dissection.

7.2 Research Component (Treatment with cetuximab and associated biopsies)

All patients will sign an informed consent for clinical and research biopsies. All biopsies will be performed by an otolaryngologist or oral surgeon who is a co-investigator of the study. The proposed tissue accrual should be adequate for all of the proposed studies.

7.2.1 Research Biopsy #1: Patients will be consented and enrolled in this study prior to this biopsy. The biopsy will be performed no more than 4 weeks prior to the loading dose of cetuximab. It will be performed under local anesthesia (in the event a patient requires further clinical staging or confirmation of diagnosis under general anesthesia, the research biopsy may be performed at that time.) This pre-treatment biopsy will be performed for research purposes if a suitable clinical biopsy specimen is not available. A clinical specimen will be suitable if the biopsy was performed within 4 weeks of the loading dose of Cetuximab.

7.2.2 Research Biopsy #2: This will be done 7 days (+/- 1 day) after the cetuximab loading dose (Day 0).

7.2.3 Normal Oral Mucosa Sampling: Baseline EGFR of the normal oral mucosa will be obtained by buccal swab or normal mucosa biopsy. If using buccal swabs, at least 3 normal oral mucosa buccal swab

samples will be acquired. This sampling will occur at both Research Biopsy #1 and Research Biopsy #2.

7.3 Cetuximab/Radiation Schedule: Cetuximab will be delivered as standard of care per the package insert.

7.3.1 Day 0: Patients will receive a single dose of cetuximab 400 mg/m^2.

7.3.2 Day 7 (+/- 1 day): Biopsy #2 will be obtained

7.3.3 Following the biopsy, within approximately 4 days, daily radiation therapy with weekly cetuximab will be initiated.

7.3.4 Patients will receive weekly cetuximab 250 mg/m2. On days of radiation therapy and cetuximab administration, cetuximab will be given prior to radiation therapy.

7.4 Cetuximab Dose Levels and Modifications

	Starting Dose	**Dose Level –1**	**Dose Level –2**
Cetuximab	400 mg/m^2 (day 0) 250 mg/m^2 (day 7 and weekly during RT)	200 mg/m^2 (weekly)	150 mg/m^2 (weekly)

NOTE: Once the dosage of cetuximab has been decreased, it will remain at that level unless further dose reductions are required, at which time all dosages will remain at that level. Dosages CANNOT be increased once a dose reduction has taken place.

7.4.1 Cetuximab Dose Modification for Hematologic Toxicity

NCI CTCAE Toxicity Grade (CTCAE v. 4.0)	**Cetuximab Dose [a,b] at Start of subsequent Cycles of Therapy**
Neutropenia	
1 (1500-1999/mm^3)	Maintain dose level
2 (1000-1499/mm^3)	Maintain dose level
3 (500-999/mm^3)	Decrease by 1 dose level with occurrence
4 (<500/mm^3)	Decrease by 1 dose level with occurrence
Neutropenic Fever[c]	Decrease by 1 dose level
Thrombocytopenia	
1 (≥75,000/mm^3)	Maintain dose level
2 (50,000- 74,999/mm^3)	Maintain dose level
3 (25,000 – 49,000/mm^3)	Decrease by 1 dose level with occurrence
4 (<25,000/mm^3)	Decrease by 1 dose level with occurrence

[a]Dose levels are relative to the starting dose in the previous cycle. Dose reductions of cetuximab below the –2 dose level will not be allowed.
[b]Provided that all the retreatment criteria are met (see section 7.4.5)
[c]One reading of oral temperature ≥38.5ºC and ANC ≤ 500

7.4.2 **Cetuximab Dose Modification for Non-Hematologic Toxicity**

NCI CTCAE Toxicity Grade (CTCAE v. 4.0)	**Cetuximab Dose[a,b,c,d]**
Fatigue (Asthenia) ≥ Grade 3	Decrease by 1 dose level
Nail changes (paronychia) Grade 2	Decrease by 1 dose level
Diarrhea Grade 3 despite maximal medical management	No dosage adjustment
Grade 3 recurrent, despite maximal medical management	Decrease by 1 dose level
Grade 4 despite maximal medical management	Decrease by 1 dose level
Headache	
≥ Grade 3	Decrease infusion rate by 50%
≥ Grade 3 despite decreased infusion rate and use of analgesic	Decrease dose by 1 dose level
Stomatitis/Mucositis	All patients are evaluated weekly. The dosage adjustments will be determined by the practicing clinicians at each visit. If a reduction is required, the dose will be decreased by 1 dose level. If > 2 dosage reductions are required, cetuximab will be discontinued.

[a]For CTCAE Grade < 2 non-hematologic toxicity not described above, maintain dose level of drug.
[b]Provided that all the retreatment criteria are met as detailed in section 7.4.5.
[c]Dose levels are relative to the previous dose. Dose reductions of cetuximab below the –2 dose level will not be allowed.
[d]In any case of cetuximab treatment delay, there will be no reloading infusion, and all subsequent treatments will be at the assigned dose level.

7.4.3 **Management of Cetuximab Hypersensitivity Reactions**

CTCAE Grade	Hypersensitivity Reaction
Grade 1	Transient rash, drug fever <380C (<100.40F),
Grade 2	Urticaria, drug fever > 380C (> 100.40F), and/or asymptomatic bronchospasm
Grade 3	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema
Grade 4	Anaphylaxis

7.4.3.1 Symptoms of hypersensitivity reactions should be managed per institutional chemotherapy infusion policy guidelines.

7.4.3.2 Study Therapy Retreatment Following Hypersensitivity Reactions: Once a cetuximab infusion rate has been decreased due to an allergic/hypersensitivity reaction, it will remain decreased for all subsequent infusions. If the subject has a second allergic/hypersensitivity reaction with the slower infusion rate, the infusion should be stopped, and the subject should receive no further cetuximab treatment. If a subject experiences a Grade 3 or 4 allergic/hypersensitivity reaction at any time, the subject should receive no further cetuximab treatment. If there is any question as to whether an observed reaction is an allergic/hypersensitivity reaction of Grades 1-4, the Principal Investigator or co-investigator should be contacted immediately to discuss and grade the reaction.

7.4.5 Cetuximab Special Instructions

If cetuximab is omitted for more than four consecutive infusions for toxicity due to cetuximab, or for an intercurrent illness (e.g., infection) requiring interruption of therapy, the subject should be discontinued from further cetuximab therapy. If toxicities prevent the administration of cetuximab, the subject may continue to receive radiation therapy.

7.4.5 Retreatment Criteria for cetuximab

Cetuximab may only be administered if all of the following criteria are met regardless of cycle, providing no criteria for discontinuation are met (see Section 10.0):

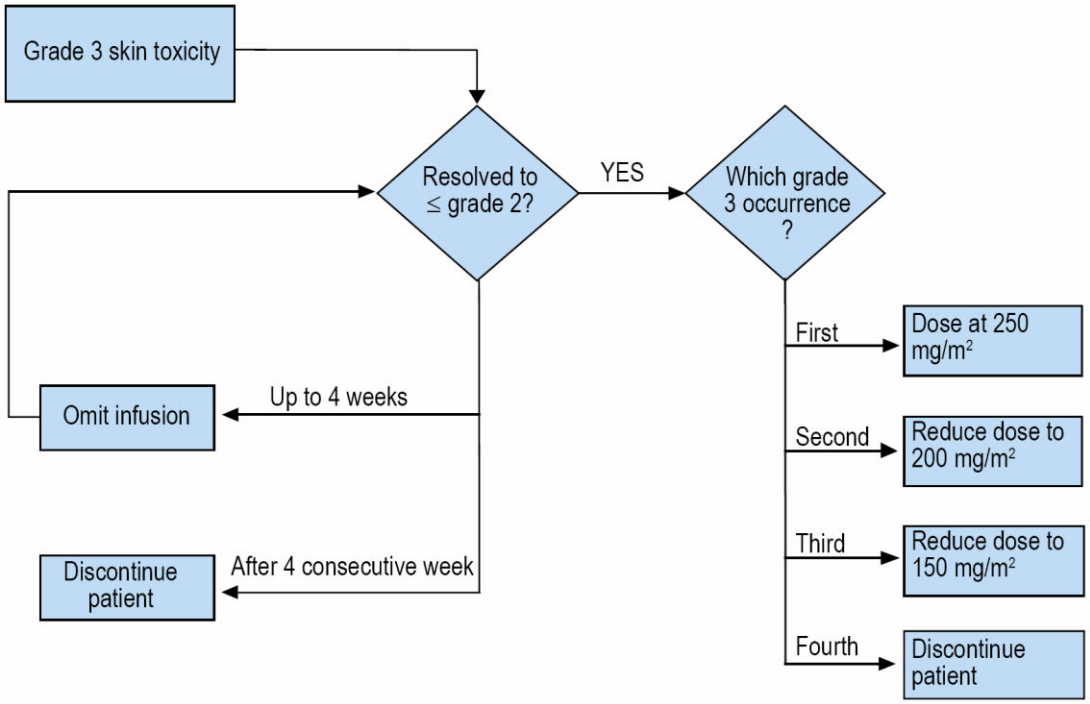
- Acne-like rash is ≤ Grade 2 (see Section 7.4.6)
- Grade 3 - 4 hematologic toxicities have resolved to ≤ CTC Grade 2

- Grade 3 - 4 non-hematologic toxicities have resolved to ≤ CTC Grade 2, (except fatigue (asthenia), anorexia and alopecia)

7.4.6 Acne-Like Rash (rash acneiform or rash maculo-papular)

The dose of cetuximab will be adjusted for Grade 3 acne-like rash. The severity of these events will be graded according to the criteria for the CTC term "rash acneiform or rash maculo-papular." The cetuximab dose alteration scheme is outlined in the figure below. If a subject experiences a Grade 3 acne-like rash (rash acneiform or rash maculo-papular), cetuximab therapy is to be held for up to four consecutive infusions (see table below). The Investigator could also consider concomitant treatment with topical and/or oral antibiotics; topical corticosteroids are not recommended. If there are subsequent occurrences of a Grade 3 acne-like rash (rash acneiform or rash maculo-papular), cetuximab therapy may again be omitted for up to four consecutive weeks. Treatment may resume with reduced doses of cetuximab if the skin toxicity has resolved to Grade 2 or less. Cetuximab dose reductions are permanent. Cetuximab will be discontinued if there is a subsequent occurrence of a fourth episode of Grade 3 acne-like rash (rash acneiform or rash maculo-papular) or there are more than four consecutive infusions held. The subject should be followed weekly until resolution of the rash. If a subject experiences a Grade 4 acne-like rash (rash acneiform or rash maculo-papular), cetuximab therapy will be discontinued.

Management of Cetuximab Acne-Like Rash



7.5 Evaluation of Response to Treatment

7.5.5 Careful evaluation of tumor extent will be recorded for both the primary tumor and regional nodes at specified intervals by the treating radiation oncologist, medical oncologist or otolaryngologist, who are co-investigators of the study.. CT-PET scans will be used at the discretion of the clinician to supplement clinical exams. However, it is not necessary for imaging abnormalities to revert to normal in order for a patient to be considered a clinical CR.

7.5.6 Biopsy of any persistent neck nodes is required at the 12-14 week post cetuximab-radiation laryngoscopy. Patients with any nodes initially > 3 cm in size who are CT-PET positive at 12-14 weeks post cetuximab-RT will undergo neck dissection. Patients whose CT-PET shows a complete response (CR) at 12 weeks post cetuximab-RT, will undergo clinical observation.

7.5.7 Diagnostic CT-PET scans may be obtained prior to scheduled endoscopies for tumor assessment.

7.5.8 Outpatient clinical examinations will be performed as comparable to clinical practice: at approximately 1 month following completion of RT and then approximately every 2 months during years 1 and 2. Clinical examinations will be performed by medical oncology, radiation oncology, and/or surgical oncology. To meet the examination requirements, patients need to be seen by at least one discipline (but not all three) during the aforementioned time intervals.

7.6 Evaluation of Toxicity and Quality of Life

7.6.5 Validated xerostomia-related and general quality of life questionnaires (Appendix C), and Common Toxicity Criteria Adverse Events (CTCAE, v.4) observer related items, will be collected before, during and after radiation therapy as indicated in the study calendar. As part of data collection related to the quality of life measures, pre and post-treatment employment status will also be recorded. Patients may be asked for this information during a clinic visit or contacted via phone. The study team will attempt to obtain this information from all enrolled patients.

8.0 Study Calendar

Assessment	Pre-Treatment*	Day 0 Loading Dose	Day 7	Weekly during RT†	1 mo post RT[2]	12-14 wks post RT[2]	Follow Up Years 1 and 2[2]
H&P/Physician Evaluation[1]	X			X	X	X	X
Research Biopsy	X**		X				
Dental Evaluation[3]	X						
COMP, Mg	X			X		X	
CBCP with diff	X			X		X	
CT-PET[3]	X					X	
CT or MRI of head/neck	X						
Toxicity Evaluation	X			X	X	X	X
QOL Questionnaire	X			end of treatment	X	X	X
Cetuximab		X		X			

* The pre-treatment period is prior to the administration of the cetuximab loading dose. The pre-treatment toxicity evaluation and QOL Questionnaire may be completed at the time of consent. When a patient is determined eligible and enrolled, data will be used. If patient is not eligible or not enrolled, the data will not be used and the subject will be deemed a screen failure. The forms will be kept with the Informed Consent in the study record.

**** Research biopsy #1 must be within 4 weeks of loading dose (Day 0)**

† Radiation therapy will start within approximately 4 days following Day 7 (+ or – a day) biopsy

[1]Evaluations pre-treatment are by Radiation Oncology, Otolaryngology, and Medical Oncology. Evaluations weekly during RT are generally by Radiation Oncology via standard on-treatment notes. Evaluations in follow up will be by Radiation Oncology, Otolaryngology, and/or Medical Oncology. It is not necessary for patients to be seen by more than one discipline at each follow up visit.

[2]Follow up: Examinations will be performed as comparable to clinical practice: at approximately 1 month following completion of RT and then approximately every 2 months during years 1 and 2. At approximately 12-14 weeks post-RT, patients will have clinical restaging performed for disease evaluation. A PET or MRI is acceptable for this restaging. Toxicity Evaluation and QOL Questionnaires will be collected at each of these visits. Biopsies, labs and imaging studies will be performed as clinically indicated. For years 3 - 5, all follow up will be as clinically indicated and patients will only be followed for disease status and survival.

[3] Pre-treatment PET-CT and pre-treatment dental evaluation preferred, but these will be ordered at the discretion of the treating physician.

9.0 Measurement of Response

9.4 Tumor Clearance

A primary clinical endpoint of this study is progression-free survival. A patient will be considered to have a complete response if there is no measurable or palpable tumor either on clinical or radiographic (CT-PET scan or MRI) examination assessed within approximately 3 months after the completion of treatment. Complete response will

be defined as complete disappearance of disease (in which case 9.2 will apply to assess relapse) or residual radiographic abnormality (which cannot be safely biopsied) that is not considered to be tumor (in which case section 9.3 will apply to assess progression).

9.5 Local or Regional Relapse

Relapse is defined as reappearance of tumor after complete response. If possible, relapse should be confirmed by biopsy.

9.6 Local or Regional Progression

Progression is defined as an estimated increase in the size of the tumor (product of the perpendicular diameters of the two largest dimensions) of greater than 25%, taking as reference the smallest value of all previous measurements or appearance of new areas of malignant disease.

9.7 Distant Metastasis

Clear evidence of distant metastases (lung, bone, brain, etc.); biopsy is recommended where possible. A solitary, spiculated lung mass/nodule is considered a second primary neoplasm unless proven otherwise.

9.8 Second Primary Neoplasm

Tumor reappearing with the initial and immediate adjoining anatomical region of the primary will be considered local recurrence. Multiple lung nodules/masses are considered distant metastases from the index cancer unless proven otherwise.

10.0 Criteria for Discontinuation of Treatment

In the absence of treatment delays due to adverse events, treatment may continue through completion of concurrent chemoradiotherapy and consolidation chemotherapy or until one of the following criteria applies:

- Local-regional disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

If treatment is interrupted due to a non-dose-limiting adverse event or any reason other than toxicity, such as a holiday, bad weather, or a transportation problem, the duration of therapy will be extended accordingly. If a patient misses a day of radiation and chemotherapy, then the weekly chemotherapy should be delivered the next day and the missed radiation fraction will be given after the completion of planned treatments.

Patients who exhibit local-regional tumor progression will discontinue all study procedures and will be medically managed. For the purposes of the research, they will continue to be followed for toxicity and survival. These patients may be treated with other agents.

11.0 Drug Information: Cetuximab

The product will be provided from commercial supply.

11.4 Formulation

Cetuximab is an anti-EGFR receptor humanized chimeric monoclonal antibody. Cetuximab is expressed in SP2/0 myeloma cell line, grown in large scale cell culture bioreactors, and purified to a high level purity using several purification steps

including protein A chromatography, ion exchange chromatography, low pH treatment, and nanofiltration. Cetuximab is not known to be a vesicant.

11.5 Supply

The product is formulated to 2 mg protein/mL with phosphate buffered saline, pH 7.2 ± 0.2 and aseptically filled into sterile glass vials, 100 mg per 50 cc vial, and stored as a liquid at 2 to 8º C. Each vial contains the following active and inactive ingredients per 1.0 ml: 2 mg of cetuximab, 145 nmol/L sodium chloride, and 10 mmol/L sodium phosphate.

11.6 Safety Precautions

Appropriate mask, protective clothing, eye protection, gloves and Class II vertical-laminar-airflow safety cabinets are recommended during preparation and handling.

11.7 Preparation and Administration:

11.7.5 Cetuximab is provided by ImClone as an injectable solution, in single-use, ready-to-use 50-mL vials containing 2 mg/mL of product. Cetuximab requires no dilution. Cetuximab should not be mixed with or diluted with other drugs or solutions for infusion such as 5%-glucose.

11.7.6 The dose and volume of the study drug to be infused are dependent upon the patient's actual BSA. The infusion rate must never exceed 10 mg/minute (5 mL/minute). The dose may subsequently be reduced for individual patients, depending on a patient's toxicity. For the duration that patients are on cetuximab therapy, adverse event monitoring should be done continuously. Patients will be evaluated for adverse events at each visit and are to be instructed to call their physician to report any adverse events between visits.

11.7.7 Cetuximab may be administered via a gravity drip, infusion pump, or syringe pump. Cetuximab administration requires an in-line low protein-binding 0.22 micron filter. Note: one filter per dose should be sufficient, but further filters can be used if a filter becomes blocked.

Administration via Infusion Pump or Gravity Drip

Calculate the appropriate volume of cetuximab based on the dose and using an appropriate sterile syringe (min 50 mL) draw up the required volume from the vial(s). Add the cetuximab into a sterile evacuated container or bag (glass administration containers are not recommended). Do not shake. Attach an infusion line with a low protein binding 0.22 micron in-line filter. Use a gravity drip or an infusion pump for administration. Set and control the rate as noted above and infuse the whole dose.

Administration via Syringe Pump

Calculate the appropriate volume of cetuximab based on the dose and using an appropriate sterile syringe (min 50 mL) draw up the required volume from the vial(s). Do not shake. Remove the needle, and put the syringe into the syringe pump. Attach tubing with a low protein-binding 0.22 micron filter. Set and control the rate as described above. Make sure that the whole dose has been infused.

Studies have been conducted to demonstrate the compatibility of cetuximab drug product with various infusion systems. Some

examples of materials, IV containers, infusion sets, and filters tested and recommended for use with cetuximab are listed below. For further examples of approved materials, please see the Investigator Brochure.

Recommended IV Containers

- IntraVia™ IV Bag with PVC Ports, Model No. 2J8002 (Baxter Healthcare Corporation)
- EVA™ IV Bag, Model No. 2B8152 (Baxter Healthcare Corporation)
- LifeCare™ IV Bag, Model No. 7951-12 (Abbott Laboratories)

Recommended Infusion Sets

- Vented Continu-Flo Solution Set™, Model No. 2C6541s (Baxter Healthcare Corporation) to be used with an in-line filter set, Model No. 2679 (Abbott Laboratories)
- Vented Paclitaxel Set™ with 0.22-µm downstream high-pressure in-line filter, Model No. 2C7553 (Baxter Healthcare Corporation)

Recommended Filters

- Vented Continu-Flo Solution Set™, Model No. 2C6541s (Baxter Healthcare Corporation) to be used with an in-line filter set, Model No. 2679 (Abbott Laboratories)
- Intrapur Plus (B. Braun AG) reference number 409 9800
- Poly-lined filtered Extension set (Alaris Medical Systems) reference number C20350

11.7.8 Normal saline should be use to clear the infusion set of residual cetuximab. The delivered drug product is > 95% for all recommended infusion sets when flushed with 50 mL of normal saline. Use a separate line for cetuximab infusion.

11.7.9 Storage Requirements/Stability: Cetuximab must be stored under refrigeration at +2˚C to +8˚C (+36˚F to +46˚F). DO NOT FREEZE CETUXIMAB. Drug supplies must be kept in a secure, limited access storage area under the recommended storage conditions. Once cetuximab is removed from the vial, the recommended maximum storage time in the infusion container or syringe is 8 hours at room temperature or 12 hours in the refrigerator.

11.8 Adverse Events:

- Hematologic: Leukopenia
- Gastrointestinal: Nausea, vomiting, diarrhea, anorexia, mucous membrane disorder, stomatitis, reduced kidney or liver function
- Dermatologic: Rash, acne, dry skin, pruritus
- Circulatory: Deep vein thrombosis
- Neurological: Confusion, disorientation, seizure, coma; rarely, encephalitis
- Allergy: Allergic reaction, anaphylactoid reaction
- Other: Asthenia, fever, dyspnea, headache, chills, nail disorder, myalgia, arthralgia

12.0 Radiation Therapy

12.4 General Considerations:

All the patients in this study will receive definitive radiotherapy. Definitive radiotherapy will begin one week following the administration of the loading dose of cetuximab.

12.5 Radiation Fields:

The treatment volumes will be individualized for each patient depending upon the extent of disease. Tumor volumes will be outlined on the planning CT scans with the aid of CT-PET to ensure adequate irradiation of the pre-cetuximab tumor volume. Treatment techniques will aim at adequate irradiation of the clinical and the sub-clinical disease. The therapy goals, specifying the intended doses to the primary tumor and lymph node metastases, will be detailed in the therapy chart. A CT-based display of the isodoses will be recorded, such that it will be feasible to assess whether the intended (prescribed) isodoses cover the targets adequately.

12.6 Doses:

Tumor doses will be expressed in Gy. The prescribed doses should encompass the targets. Treatment plans will be generated demonstrating adequate coverage of the target volume. The dose across the target volume should not vary by more than +/- 10% of the prescribed dose. When an anterior-posterior low neck field is treated, the dose will be prescribed to 3cm depth. For electron beam treatments, the dose will be prescribed to the depth at which maximum dose is obtained (Dmax). When treating the posterior cervical nodes, either six or nine MeV electrons may be used. Treatment will be delivered daily, five days per week, 2.0 Gy per fraction. Total dose to gross disease will be 70 Gy and subclinical disease dose will be 50-60 Gy. Intensity Modulated Radiation Therapy (IMRT) will be used to decrease dose to the normal critical structures when appropriate, as determined by the treating radiation oncologist.

12.7 Immobilization and Positioning:

All patients will be treated in a position that affords maximal daily reproducibility. Commonly, it will be in the supine position. Immobilization devices such a head masks or bite blocks are mandatory to ensure that target volumes are adequately treated. Cradles or arm restraints may be needed to allow adequate exposure of the lower neck area in selected patients.

12.8 Simulation and CT Scanning:

All patients will undergo simulation including a CT scan. Treatment planning CT scans with or without contrast will be obtained on each patient prior to the first week of cetuximab to ensure adequate radiation of the pre-cetuximab tumor extent.

12.9 Technical Factors:

Equipment - Megavoltage equipment with a source to skin distance of 100cm (or source-axis distance), or greater, will be used. Megavoltage machines with an energy equal to 6 MV photons will be used, rarely higher energy may be used if necessary.

12.10 Treatment Planning:

CT based planning may be used for the total course of radiation, especially if necessary for parotid sparing purposes. Otherwise, the final boost may be planned or the full course of radiation may be planned using orthogonal simulation fields alone,

using the information from the pre-cetuximab planning CT for verification of the adequacy of the radiation fields.

12.11 Treatment Interruptions:

It is expected that the entire treatment for definitive irradiation will be completed in about 7-8 weeks. Treatment interruptions due to symptomatic mucositis or skin reactions are rare. In the case of severe mucositis, preventing appropriate nutritional intake, a gastric tube or dobhoff tube will be inserted and radiation will continue uninterrupted at the discretion of the treating physician. Weight will be recorded weekly in the Radiation Oncology chart. If the patient's weight loss exceeds 10% of the initial weight or if the patient is malnourished before radiation, a feeding tube may be inserted by an appropriately trained physician (surgeon, gastroenterologist, interventional radiologist) as defined by clinical practice.

13.0 EGFR analysis (General Laboratory Guidelines)

The primary goal of this analysis is to determine whether cetuximab given at the clinically recommended dose, will inhibit EGFR phosphorylation and down-stream signaling effects within the tumor as well as the normal mucosa following therapy. In addition, the activation of EGFR and various down-stream signaling markers will be measured using immunoblotting as well as immuno-histochemical analysis in both normal and tumor samples at baseline and post-treatment.

13.4 Pre-Cetuximab tumor

The initial pre-treatment punch-biopsy specimen (about 3x3x3 mm) of the tumor will be collected in the presence of a laboratory personnel and will be immediately frozen in dry-ice ethanol bath in Tissue-Tek O.C.T Compound (Sakura Finetek,Torrance, CA) to ensure the integrity of tissue and phosphoproteins for both immunoblotting and immunohistological analysis of samples. We will be able to take 3 cores for TMA construction from a single 3x3x3 mm biopsy and will expect to have enough material to get about 30-50 4-5 micrometers thick sections for immunohistochemical analysis.

13.5 Method of Assessment of EGFR Expression in Tumor After Cetuximab Loading Dose

At the time of the second biopsy, four core biopsies from different areas of the tumor as well as swab of the normal appearing mucosa will be obtained. Four core biopsies will be processed with one core used in high-throughput immunoblot analysis, one core will be fixed in formalin to be embedded in paraffin, one core will be frozen and fixed in Tissue-Tek OCT for immunofluorescence analysis and one core will be used for RNA and DNA isolation using Trizol. EGFR phosphorylation analysis using immunoblotting and immunohistochemistry will be performed in the surgical specimen and immunofluorescence analysis will also be performed if sufficient tissue is available using standard methods. Interpretations of the staining will be performed according to the standard guidelines. The EGFR and critical associated down-stream signaling pathways will be analyzed post- induction therapy, including p-EGFR and total EGFR, p-ERK1/2, p-AKT, p27, Ki-67, and p-STAT3, and other markers that we may discover in our preclinical studies. EGFRvIII expression and p53 mutation analysis will be assessed on cDNA obtained from total RNA converted with RT-PCR. Genomic DNA from the tumor biopsy will be used to test for HPV presence and type as carried out by PCR-mass spect using the AttoSense assay at SensiGen, LLC.

14.0 Other Therapy

All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol and documented as concomitant medication.

15.0 Informed Consent

15.4 All patients with squamous carcinoma of the head and neck, who are candidates for definitive radiation, will be screened for participation in this study.

15.5 Alternative treatment options include chemoradiation, radiation therapy alone, or palliative chemotherapy.

15.6 Patients who meet the inclusion criteria will be approached for possible participation in this study. The nature of the investigation will be described to the patient including the risks and side effects of study treatments, the potential benefit of the study to themselves and others, the time commitment and frequency of patient visits and the clinical evaluations they will be required to undergo. The patient will then have the opportunity to ask questions. The patient will be given the IRB approved informed consent form for consideration. Each patient will be allowed to read (or have read to them) the informed consent form and understand before discussing consent with the investigator. If consent to participate is granted, the patient's signature will be obtained on the informed consent form. The original form will be kept in the patient's research chart and a copy will be given to the patient.

16.0 Patient Registration

Patient registration for this trial will be centrally managed by the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log provided by the Clinical Trials Office.

It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the Clinical Trials Office. After patient eligibility has been determined, a copy of the completed Eligibility Worksheet together with all the pertinent de-identified source documents will be submitted by the requesting site to the Clinical Trials Office, either by fax or by email to [redacted]

A Multi-Site Coordinator of the Clinical Trials Office, who acts as the registrar, will review the submitted documents and process the registration. Sites should inform the Multi-Site Coordinator of a potential registration by 5 p.m. on the day prior to registration. Same day registrations cannot be guaranteed.

An email will be sent by the registrar to the requesting site registrar to confirm patient registration and to provide the study identification number that has been assigned to the patient. In addition, a copy of the completed Section Two of the Eligibility Worksheet signed and dated by the registrar, will be faxed back to the requesting site registrar.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These

patients will not have study identification number assigned to them, and will not receive study treatment

17.0 Adverse Drug Events

1.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. Data on adverse events will be collected from the time of the initial investigational agent administration through the two year study. Serious Adverse Events (SAEs) will continue to be followed until resolution or clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE. The definitions of AEs and SAEs are given below. It is the responsibility of the principal investigator to ensure that all staff involved in the trial is familiar with the content of this section.

Any medical condition or laboratory abnormality with an onset date before agent administration is considered to be pre-existing in nature. Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history.

All adverse events occurring from the initial investigational agent administration through the two year study calendar must be recorded as an adverse event in the patient's source documents regardless of frequency, severity (grade) or assessed relationship to the investigational agent/intervention. In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins taking the investigational agent/intervention is also considered an adverse event. All moderate events (grade 2) and hematologic serious events (grade 3) due to the patient's cancer or the treatment which are common toxicities and expected will be excluded from this data collection. These will be noted in the patient's medical records.

All adverse events specified in the Case Report Form Completion Guidelines will be recorded in the study database (Velos)

17. 1 Definitions

Adverse event

Adverse event means any untoward medical occurrence associated with the use of a medical treatment or procedure regardless of whether or not considered related to the medical treatment or procedure.

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, without any judgment about causality.

Unexpected

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Serious Adverse Event
An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor (UNIVERSITY OF MICHIGAN), it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Previously planned (prior to signing the informed consent form) surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient’s medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE.

Life-threatening
An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

17.2 Adverse Event Characteristics

CTCAE term

Adverse events (AEs) will use the descriptions and grading scales found in the revised National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE).

A copy of the CTCAE version 4 can be downloaded from the CTEP home page: http://ctep.info.nih.gov/reporting/ctc.html

Attribution of the AE:

The investigator is responsible for assignment of attribution.
Definite – The AE is clearly related to the investigational agent.
Probable – The AE is likely related to the investigational agent.
Possible – The AE may be related to the investigational agent.
Unlikely – The AE is doubtfully related to the investigational agent.
Unrelated – The AE is clearly NOT related to the investigational agent.

18.0 Reporting Procedures

Serious adverse Events (SAEs) should be reported to the Principal Investigator and Multi-Site Coordinator within 48 hours of awareness of the event. SAEs will be reported using the CTO SAE form in Velos.

Principal Investigator: Shruti Jolly, MD
[redacted]

Multi-Site CoordinationFax [redacted]
Email:[redacted]

Follow-up information must also be reported within 48 hours of receipt of the information.

Investigators must report SAEs to their local Institutional Review Board per their institutions guidelines.

The Multi-Site Coordinator will be responsible for distributing all SAE reports to participating institutions.

18.1 Exceptions to SAE Reporting

The following adverse events are excluded from SAE reporting:

- Hospitalization secondary to expected cancer morbidity
- Admission for palliative care or pain management
- Planned hospitalizations for surgical procedures either related or unrelated to the patient's cancer.
- Emergency Department visits not related to study treatment

19.0 Data and Safety Monitoring

The Data and Safety Monitoring Board (DSMB) of The University of Michigan Comprehensive Cancer Center (UMCCC) is the DSMB for this study. This committee is responsible for the review and monitoring the study's scientific progress, accrual rate and any serious adverse events.

Each participating site is required to have its own Data and Safety Monitoring Committee (DSMC) for the study. This committee will be composed of the local site principal investigator, site co-investigator(s), site data manager or study coordinator and other members of the study staff involved in the conduct of the trial. During the committee's bimonthly meeting, the principal investigator will discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

These meetings are to be documented by the site data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the site principal investigator or co-investigator. Each site is required to submit the completed DSMR to the Multi-Site Coordinator at the University of Michigan Clinical Trials Office on a quarterly basis together with other pertinent documents.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of both the sites data manager or study coordinator and the site principal investigator [or co-investigator]. These reports are to be sent to the University of Michigan Clinical Trials Office within 7 calendar days of awareness of the event and on a quarterly basis with the Protocol Specific Data and Safety Monitoring Report.

The Clinical Trials Office is responsible for collating all the Data and Safety Monitoring Reports from all the participating sites, and providing the information to the Data Safety Monitoring Board.

20.0 Clinical Monitoring Procedures

Clinical studies coordinated by The University of Michigan Comprehensive Cancer Center (UMCCC) must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements.

This study will be monitored by a representative of the Clinical Trials Office (CTO) of the UMCCC. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the Clinical Trials Office. This will be done as an actual site visit; teleconference, videoconference, or web-based meeting after the site has been given access to the study database and assembled a study reference binder. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting. Study–related questions or issues identified during the site initiation meeting will be followed-up by the appropriate UMCCC personnel until they have been answered and resolved.

The first annual monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The initial annual visit is not justified unless there is at least one participant enrolled on a study. At a minimum, a routine monitoring visit will be done at least once every 12 months, or once during the course of the study if the study duration is less than 12 months. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

Monitoring visits may be in the form of a site visit or a review of the documents at the CTO. During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the CTO representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The Clinical Trials Office expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner. For review of study-related documents at the CTO, the site will be required to ship or fax documents to be reviewed.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit if all of the following apply:

- No patient has signed the Informed Consent Form and has enrolled into the study
- Investigational agent has not been dispensed
- All investigational agent and materials have been returned as defined for the study or destroyed and accounted for properly.

20.1 Quality Assurance and Audits

The Data Safety Monitoring Board can request a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues. A "for cause" audit would be conducted by the Quality Assurance Review Committee (QARC) of the University of Michigan Comprehensive Cancer Center.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Clinical Trials Office that such a request has been made.

21.0 Statistical Considerations

21.1 General Description: This is a single-arm, Phase II trial to characterize the clinical outcome of standard of care, cetuximab concurrent with radiation, in a special population (head and neck cancer patients who cannot tolerate concurrent chemoradiotherapy due to advanced age, poor performance status or concurrent illness), and to determine if biomarker response to a loading dose of cetuximab is predictive of that outcome.

21.2 Number of patients: Fifty evaluable patients will be accrued to the trial, which is expected to take 36 months. A patient will be evaluable for the primary objectives if he or she undergoes Research Biopsy #1 and #2 and either completes the full course of cetuximab+RT or has cetuximab+RT stopped for toxicity. Unevaluable patients will be replaced.

21.3 Analysis plan

21.3.1 **Primary Objective 1:** Determine changes in tumor EGFR, pEGFR, downstream signaling and novel phosphoproteins following a loading dose of cetuximab in patients who are poor candidates for chemoradiation (age ≥70 years or with significant co-morbidities) and are therefore treated with cetuximab+radiation. These markers will be assessed in tumor biopsies harvested before and after the loading dose of cetuximab. The initial analysis will use descriptive and graphical statistics to determine if any of the markers require transformation prior to analysis. Markers may be assessed on a continuous, ordinal or

dichotomous scale; markers measured on a continuous scale will be analyzed via general linear models (possibly after transformation), while ordinal and dichotomous markers will be analyzed via cumulative logit and logistic regression analyses, respectively. The primary questions to be answered in this objective are: is the marker modulated by the loading dose of cetuximab; are there baseline clinical or demographic variables that are related to significant variation in biomarker response to the loading dose of cetuximab? For continuous variables, the null hypothesis that the mean value of the change (absolute or percent of baseline) equals zero will be tested by means of a single sample t-test. The second question will be answered by means of analysis of variance on baseline clinical and demographic variables. For dichotomous markers, patients will be stratified into elevated versus not elevated at baseline, and Wald tests on logistic regression parameter estimates will be used to test the null hypothesis that the proportion of patients changing state from baseline is different from 0.5, and to determine if any subset of patients defined by clinical or demographic factors have proportions significantly different from 0.5. Ordinal markers will be handled in a similar fashion.

21.3.2 **Primary Objective 2** Characterize clinical outcomes, including local recurrence, progression-free survival and overall survival in these patients, and correlate these clinical outcomes with the changes in tumor EGFR, pEGFR, downstream signaling, and novel phosphoproteins. Logistic regression and proportional hazards (Cox) regression will be used to relate clinical out (local recurrence, progression-free survival or overall survival) to changes in markers across the loading dose. All of the markers will be analyzed, but markers that are modulated by the loading dose (identified in Primary Objective 1) will be of particular interest.

21.3.3 **Primary Objective 3** *Describe the toxicity, in particular mucositis/dysphagia, of this regimen.* Adverse events will be tabulated by grade (NCI CTCAE v4), category (mucositis and dysphagia are expected to be the most common) and relatedness to treatment (not, unlikely, possibly, probably or definitely). The proportion of patients experiencing Grade 3 or worse adverse events at least possibly related to treatment will be calculated, accompanied by a 95% exact binomial confidence interval. Possible baseline demographic and clinical predictors of such toxicity may be evaluated by means of logistic regression, but, due to the low expected number of such toxicities, any such modeling will be strictly exploratory.

21.3.4 **Secondary Objective 1** *Conduct normal mucosa EGFR assessment for comparison with tumor sample.* The change in tumor EGFR level across treatment, relative to EGFR in normal mucosa, will be evaluated by means of repeated measures ANOVA.

21.3.5 **Secondary Objective 2** *Correlate HPV presence and titer with p53 status and clinical outcome.* HPV status (±) will be added to the models of Primary Objective 2 to determine if +HPV patients respond to therapy differently than -HPV patients, but, due to the limited expected number of +HPV patients, such analyses will be considered exploratory.

21.4 Justification of Design: While radiation with concurrent cetuximab has become the standard of therapy in patients with locally advanced squamous cell head and neck carcinoma, the clinical outcome of the therapy have not been systematically studied. In addition, therapy with cetuximab is expensive, and, while less toxic than platinum-based therapies, is not without toxicity, and a screen for patients from whom cetuximab would be of benefit would be desirable. Therefore, all patients on this protocol are treated the same, and the primary objectives are focused on comparing patients who do respond to patients who do not. Because this is the standard of care, and these patients have no other treatment options, there is no provision for halting the trial due to low efficacy or excess toxicity (although therapy can, of course, be stopped in individual patients if clinically warranted). The sample size is justified in terms of Primary Objective 2, specifically relating change in a continuous marker to progression-free survival. Monte Carlo simulation was used to simulate the analysis of a single biomarker related to progression-free survival. It was assumed that 50 patients were accrued over 36 months, that the median survival was 22 months, the proportional hazards regression was performed six months after the last patients was treated, and that the biomarker was normally distributed. The power of the null hypothesis of no marker effect (tested at $\alpha=0.05$) was assessed as the effect size of the marker (here, represented as the difference between the mean of the marker for patients with PFS less than the median versus the mean of the marker for patients with PFS greater than the median, divided by the standard deviation) was increased from 0 to 1.5 standard units. The result is shown in Figure 20.1, where it is seen that the power to reject the null hypothesis of no effect is at least 80% if the effect size is 1.1 standard units or greater, which would be considered a moderately large effect.

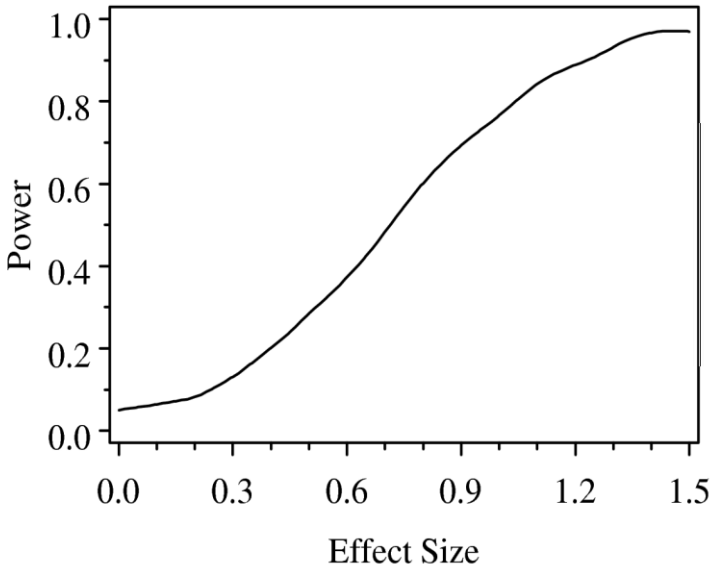


Figure 20.1 Power of proportional hazards regression for a marker that discriminates between patients with greater than versus less than median progression-free survival.

22.0 References

1. Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer. The Department of Veterans Affairs Laryngeal Cancer Study Group. *N Engl J Med* 1991;324:1685-1690.
2. Urba S, Wolf G, Eisbruch A*, et al.* Single-cycle induction chemotherapy selects patients with advanced laryngeal cancer for combined chemoradiation: a new treatment paradigm. *J Clin Oncol* 2006;24:593-598.
3. Teknos TN, Myers LL, Bradford CR*, et al.* Free tissue reconstruction of the hypopharynx after organ preservation therapy: analysis of wound complications. *Laryngoscope* 2001;111:1192-1196.
4. Eisbruch A, Thornton AF, Urba S*, et al.* Chemotherapy followed by accelerated fractionated radiation for larynx preservation in patients with advanced laryngeal cancer. *J Clin Oncol* 1996;14:2322-2330.
5. Haddad R, Tishler R, Wirth L*, et al.* Rate of complete pathological responses (pCR) to docetaxel/cisplatin/5-fluorouracil (TPF) induction chemotherapy in patients with newly diagnosed, locally advanced squamous cell carcinoma of the head and neck (SCCHN). J Clin Oncol. Vol 23; 2005. p. 5511.
6. Eisbruch A. Reducing xerostomia by IMRT: what may, and may not, be achieved. *J Clin Oncol* 2007;25:4863-4864.
7. Feng FY, Kim HM, Lyden TH*, et al.* Intensity-modulated radiotherapy of head and neck cancer aiming to reduce dysphagia: early dose-effect relationships for the swallowing structures. *Int J Radiat Oncol Biol Phys* 2007;68:1289-1298.
8. Gregoire V, De Neve W, Eisbruch A*, et al.* Intensity-modulated radiation therapy for head and neck carcinoma. *Oncologist* 2007;12:555-564.
9. Rosenthal DI, Lewin JS, Eisbruch A. Prevention and treatment of dysphagia and aspiration after chemoradiation for head and neck cancer. *J Clin Oncol* 2006;24:2636-2643.
10. J. Bourhis S, A. Le Maître, J. Pignon, K. Ang, J. Bernier, J. Overgaard, J. Tobias, M. Saunders, D. Adelstein, B. O'Sullivan. Impact of age on treatment effect in locally advanced head and neck cancer (HNC): Two individual patient data meta-analyses. 2006 ASCO Annual Meeting Proceedings. Vol 24(18S); 2006. p. 5501.
11. Bonner JA, Harari PM, Giralt J*, et al.* Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006;354:567-578.
12. Vermorken JB, Herbst RS, Leon X*, et al.* Overview of the efficacy of cetuximab in recurrent and/or metastatic squamous cell carcinoma of the head and neck in patients who previously failed platinum-based therapies. *Cancer* 2008;112:2710-2719.
13. Kwok TT, Sutherland RM. Enhancement of sensitivity of human squamous carcinoma cells to radiation by epidermal growth factor. *J Natl Cancer Inst* 1989;81:1020-1024.
14. Bonner JA, Maihle NJ, Folven BR*, et al.* The interaction of epidermal growth factor and radiation in human head and neck squamous cell carcinoma cell lines with vastly different radiosensitivities. *Int J Radiat Oncol Biol Phys* 1994;29:243-247.
15. Balaban N, Moni J, Shannon M*, et al.* The effect of ionizing radiation on signal transduction: Antibodies to EGF receptor sensitize A431 cells to radiation. *Biochimica Et Biophysica Acta-Molecular Cell Research* 1996;1314:147-156.
16. Sheridan MT, O'Dwyer T, Seymour CB*, et al.* Potential indicators of radiosensitivity in squamous cell carcinoma of the head and neck. *Radiat Oncol Investig* 1997;5:180-186.

17. Akimoto T, Hunter NR, Buchmiller L*, et al.* Inverse relationship between epidermal growth factor receptor expression and radiocurability of murine carcinomas. *Clin Cancer Res* 1999;5:2884-2890.
18. Milas L, Fan Z, Andratschke NH*, et al.* Epidermal growth factor receptor and tumor response to radiation: In vivo preclinical studies. *International Journal of Radiation Oncology Biology Physics* 2004;58:966-971.
19. Ang KK, Berkey BA, Tu X*, 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;62:7350-7356.
20. Milas L, Mason K, Hunter N*, et al.* In vivo enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody. *Clinical Cancer Research* 2000;6:701-708.
21. Nyati MK, Maheshwari D, Hanasoge S*, et al.* Radiosensitization by pan ErbB inhibitor CI-1033 in vitro and in vivo. *Clin Cancer Res* 2004;10:691-700.
22. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res* 1999;59:1935-1940.
23. Rao GS, Murray S, Ethier SP. Radiosensitization of human breast cancer cells by a novel ErbB family receptor tyrosine kinase inhibitor. *International Journal of Radiation Oncology Biology Physics* 2000;48:1519-1528.
24. Bonner JA, Raisch KP, Trummell HQ*, et al.* Enhanced apoptosis with combination C225/radiation treatment serves as the impetus for clinical investigation in head and neck cancers. *J Clin Oncol* 2000;18:47S-53S.
25. Bianco C, Bianco R, Tortora G*, et al.* Antitumor activity of combined treatment of human cancer cells with ionizing radiation and anti-epidermal growth factor receptor monoclonal antibody C225 plus type I protein kinase A antisense oligonucleotide. *Clin Cancer Res* 2000;6:4343-4350.
26. Shintani S, Li C, Mihara M*, et al.* Enhancement of tumor radioresponse by combined treatment with gefitinib (Iressa, ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, is accompanied by inhibition of DNA damage repair and cell growth in oral cancer. *Int J Cancer* 2003;107:1030-1037.
27. Solomon B, Hagekyriakou J, Trivett MK*, et al.* EGFR blockade with ZD1839 ("Iressa") potentiates the antitumor effects of single and multiple fractions of ionizing radiation in human A431 squamous cell carcinoma. Epidermal growth factor receptor. *Int J Radiat Oncol Biol Phys* 2003;55:713-723.
28. Saleh MN, Raisch KP, Stackhouse MA*, et al.* Combined modality therapy of A431 human epidermoid cancer using anti-EGFr antibody C225 and radiation. *Cancer Biotherapy and Radiopharmaceuticals* 1999;14:451-463.
29. 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;6:2166-2174.
30. Harari PM, Huang SM. Modulation of molecular targets to enhance radiation. *Clin Cancer Res* 2000;6:323-325.
31. Li J, Lin ML, Wiepz GJ*, et al.* Integrin-mediated migration of murine B82L fibroblasts is dependent on the expression of an intact epidermal growth factor receptor. *J Biol Chem* 1999;274:11209-11219.
32. Raben D, Helfrich B, Chan DC*, et al.* The effects of cetuximab alone and in combination with radiation and/or chemotherapy in lung cancer. *Clin Cancer Res* 2005;11:795-805.

33. Chung KY, Shia J, Kemeny NE*, et al.* Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol* 2005;23:1803-1810.
34. Elie C, Geay JF, Morcos M*, et al.* Lack of relationship between EGFR-1 immunohistochemical expression and prognosis in a multicentre clinical trial of 93 patients with advanced primary ovarian epithelial cancer (GINECO group). *Br J Cancer* 2004;91:470-475.
35. J. Delord J, Thomas S, Benlyazid A*, et al.* Neo-adjuvant treatment with erlotinb in squamous cell carcinoma head and neck (SCCHN) patients before surgery provides an opportunity to find predictive factors of response. Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24; 2006. p. 5513.
36. Tsao MS, Sakurada A, Cutz JC*, et al.* Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005;353:133-144.
37. Khambata-Ford S GC, Meropol NJ, Basik M, Harbison CT, Wu S, Wong TW, Huang X, Takimoto CH, Godwin AK, Tan BR, Krishnamurthi SS, Burris HA, Poplin EA, Hidalgo M, Baselga J, Clark EA, Mauro DJ. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *Journal of Clinical Oncology* 2007;25:3230-3237.
38. Ruiz-Godoy LM G-CC, Gonzalez NEH, Suchil BL, Perez-Cardenas E, Sanchez-Perez Y, Suarez-Roa ML, Meneses A. Mutational analysis of K-ras and Ras protein expression in larynx squamous cell carcinoma. *Journal of Experimental & Clinical Cancer Research* 2006;25:73-78.
39. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J Clin Oncol* 2003;21:2787-2799.
40. Sok JC, Coppelli FM, Thomas SM*, et al.* Mutant epidermal growth factor receptor (EGFRvIII) contributes to head and neck cancer growth and resistance to EGFR targeting. *Clin Cancer Res* 2006;12:5064-5073.
41. Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature* 2003;422:198-207.
42. Oda Y, Nagasu T, Chait BT. Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome. *Nature Biotechnology* 2001;19:379-382.
43. Olsen JV, Blagoev B, Gnad F, et al. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 2006;127:635-648.
44. Blagoev B, Kratchmarova I, Ong SE, et al. A proteomics strategy to elucidate functional protein-protein interactions applied to EGF signaling. Nature Biotechnology 2003;21:315-318.
45. Chun PY, Feng FY, Scheurer AM, et al. Synergistic effects of gemcitabine and gefitinib in the treatment of head and neck carcinoma. Cancer Res 2006;66:981-988.
46. Feng FY, Lopez CA, Normolle DP, 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;13:2512-2518.

23.0 Appendix

Appendix A

KARNOFSKY PERFORMANCE SCALE (KPS)

100	Normal; no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some sign or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization is indicated, although death not imminent
20	Very sick; hospitalization necessary; active support treatment is necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

Appendix B

Sample Collection and Analysis

Both pre- and post-treatment punch-biopsy specimens (about 3x3x3 mm) of the tumor will be collected and immediately placed in ice-cold saline containing a cocktail of protease (Roche Diagnostic Co., Indianapolis, IN) and phosphatase inhibitors (Sigma, St. Louis, MO). The sample will be then divided into three parts:

1. The first part will be fixed in formalin and will be used for the construction of a tissue microarray.
2. The second part will be homogenized and total proteins will be directly extracted using Laemmli buffer for immunoblotting.
3. The third part will be used for DNA and RNA extraction for assessment of HPV, p53 status and EGFRvIII expression.

In addition, Tunnel assay will also be performed to analyze the apoptosis in all the samples under investigation.

High-throughput immunoblotting and isolation of RNA and DNA for genetic studies:

Tumor samples are collected and immediately stored in Allprotect Tissue Reagent from Qiagen. They are then divided and one portion is homogenized for protein extraction 45, 46. A total of 200 µg total protein will be subjected to electrophoresis on a 2D 4 to 12% bis-tris precast gel (Invitrogen, Carlsbad, CA) and transferred onto a PVDF membrane. A Miniblotter 28 dual system (Immunetics, Cambridge, MA) will be used to probe all the antibodies in duplicate. After incubating the membrane with different antibodies overnight, membranes will be washed and probed with horseradish peroxidase conjugated IgG (Cell Signaling Technology, Beverly, MA), diluted 1:25,000 in TBST for 1 hr at room temperature; the antigen–antibody complexes will be visualized by enhanced chemiluminescence (ECL-Plus; GE Biosciences, Piscataway, NJ). The films then will be scanned, and the bands analyzed using NIH ImageJ software. Proteins to be assessed include phospho and total forms of EGFR, AKT, Src, STAT3, in addition to total p27, PARP, Bcl-XL and novel proteins for which we have either commercially available or custom-raised antibodies. Isolation of DNA and RNA will be carried out using DNAeasy and RNAeasy (both from Qiagen) on the remaining sample according to the manufacturers instructions.

Apoptosis Determination:

TUNEL assays will be performed using the In Situ Cell Death Detection Kit (Roche Molecular Diagnostics, Laval, Quebec, Canada). Slides will be evaluated by two investigators and three areas from each core will be identify based as -, +, and +++ based on relative staining intensity. These selected areas will be lifted to build a tissue micro-array for the analysis of the specific effects of treatment and to understand the mechanism of action of the cell death.

Immunohistochemistry and Scoring of TMA:

Antigen is retrieved from formalin-fixed tumor sections, in citrate buffer using a pressure cooker. Standard procedures are used for immunofluorescence. One high-density TMA will be constructed using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD, USA) at the core facility at the University of Michigan using standard methods. Tissue cores from the regions of interest (tumor

tissues, before and after treatment) will be targeted for transfer to the recipient array block. At least three 0.6 mm diameter replicate tissue cores will be sampled from each subregion of the selected sample. The final TMA will consist of approximately 200 cores. After construction, 4 µm sections will be cut, and hematoxylin and eosin staining will be performed on the initial slide to verify the histology. Serial 4 µm sections will be further cut and transferred to positively charged slides, these sections will be subjected to standard immunohistochemical staining procedures. Use of this established core enables the investigators to have access to over 200 commercially available antibodies that have been tested and validated for immunohistological use. Any antibodies not previously validated will be tested on slides made from "test arrays". These are tissue microarrays prepared from multiple tissue sources (e.g. normal mucosa, skin, brain, lymph nodes, gut, lung, spleen, liver, kidney etc., plus corresponding neoplastic tissues) created by the UM Cancer Center Tissue Procurement Core and available to the investigators. The stained test slides will be reviewed by certified pathologist for the expected pattern and appropriate intensity. The information about expected and appropriate staining for each antibody will be discussed with the other investigators to confirm the scoring criteria. The TMAs will be then stained by the staff at the Tissue Core. Each tissue core will be scored by a board certified oral and maxillofacial pathologist who is blinded as to the identity of the cores. Each tissue core will be scored for intensity of SCC cells staining: 1, undetectable; 2, weak; 3, moderate; 4, strong. An example of TMA cores is shown in Figure 2. Data analysis will be performed by Dr. Normolle. Multiple TMA core measurements from the same subject will be averaged. This average score in its continuous scale will be used in all analyses.

Immunofluorescence studies:

Immunofluorescence studies are semi-quantitative and are to be used if available antibodies do not perform well for quantitative immunohistochemical analysis. For this purpose high-resolution grey scale images are captured using a fluorescent microscope equipped with a Hamamatsu camera (Hamamatsu, Japan). The relative intensities of fluorescence are obtained using Wasabi software (Bridgewater, N.J).

Processing of the score data and statistical analysis:

All the intensities as obtained by Wasabi software will be entered into an excel spreadsheet. The spreadsheets will be then processed by using the software TMA-Deconvoluter 1.06, Cluster, and TreeView programs adapted for TMA analysis. The processed score data will be then analyzed with the SPSS for Windows statistical software package (SPSS version 11; SPSS, Chicago, IL).

Appendix C: Quality of Life Questionnaires

Quality of Life Questionnaire

Study #_____
Initials_____
Date of questionnaire_____

Each of the following items lists different numbered statements. Think about what each statement says, then place a circle around the one statement that most closely describes how you have been feeling during the ***past*** week, including today. Please circle only ***one*** statement for each item.

I. PAIN (General)

A. General
10 I have no pain.
20 There is mild pain not needing medication.
30 I have moderate pain--requires regular medication (codeine or non-narcotic).
40 I have severe pain controlled only by narcotics.
50 I have severe pain not controlled by narcotics.

B. Mouth
10 I have no pain in my mouth.
20 I have mild pain but it is not affecting my eating.
30 I have moderate pain which is affecting my eating.
40 I have severe pain and need medication in order to eat.
50 I have severe pain and cannot eat even with the medication.

C. Throat
10 I have no pain in my throat.
20 I have mild pain but it is not affecting my eating.
30 I have moderate pain which is affecting my eating.
40 I have severe pain and need medication in order to eat.
50 I have severe pain and cannot eat even with the medication.

II. DISFIGUREMENT

10 There is no change in my appearance.
20 The change in my appearance is minor.
30 My appearance bothers me but I remain active.
40 I feel significantly disfigured and limit my activities due to my appearance.
50 I cannot be with people due to my appearance.

III. ACTIVITY

10 I am as active as I have ever been.
20 There are times when I can't keep up with my old pace, but not often.
30 I am often tired and I have slowed down my activities although I still get out.
40 I don't go out because I don't have the strength.
50 I am usually in a bed or chair and don't leave home.

IV. RECREATION/ENTERTAINMENT

10 There are no limitations to recreation at home and away from home.
20 There are a few things I can't do but I still get out and enjoy life.
30 There are many times when I wish I could get out more but I'm not up to it.
40 There are severe limitations to what I can do, mostly I stay home and watch T.V.
50 I can't do anything enjoyable.

V. EMPLOYMENT

10 I work full time.
20 I have a part time but permanent job.
30 I only have occasional employment.
40 I am unemployed.
50 I am retired (circle one below)
51 not related to cancer treatment
52 due to cancer treatment

VI. EATING

A. Chewing
10 I can chew as well as ever.
20 I have slight difficulty chewing solid foods.
30 I have moderate difficulty chewing solid foods.
40 I can only chew soft foods.
50 I cannot chew soft foods.

B. Swallowing
10 I swallow normally
20 I cannot swallow certain solid foods.
30 I can only swallow soft foods.
40 I can only swallow liquid foods.
50 I cannot swallow.

VII. SALIVA

A. Amount
10 I have a normal amount of saliva
20 I have a mild loss of saliva
30 I have a moderate loss of saliva.
40 I have a severe loss of saliva.
50 I have no saliva.

B. Consistency

10 My saliva has normal consistency.
20 My saliva is slightly thicker.
30 My saliva is moderately thicker.
40 My saliva is extremely thicker.
50 I have saliva that dries in my mouth and/or on my lips.

VIII. TASTE

10 I can taste food normally.
20 I can taste most foods normally.
30 I can taste some foods normally.
40 I can taste few foods normally.
50 I cannot taste any foods normally.

IX. SPEECH

10 My speech is the same as always.
20 I have difficulty with saying some words, but can be understood over the phone.
30 I have moderate difficulty saying some words, and cannot use the phone.
40 Only family and/or friends can understand me.
50 I cannot be understood.

X. MUCUS OR PHLEGM

A. Amount

10 I have a normal amount of mucus.
20 I have a mild amount of mucus
30 I have a moderate amount of mucus.
40 I have a severe amount of mucus.
50 I have no mucus.

B. Consistency

10 My mucus has normal consistency
20 My mucus is slightly thicker
30 My mucus is moderately thicker
40 My mucus is extremely thicker
50 I have no mucus

Comments:_____
_____

Patient Name _____ Reg No. _____
Hospital _____

Date of Questionnaire _____

Below are several questions that will help describe the dryness in your mouth and how that dryness affects your daily life. Please encircle the number that corresponds to your condition during the last week in each of the following questions:

1. Rate the discomfort of our dentures due to dryness (if you do not wear dentures please check _____)

0 1 2 3 4 5 6 7 8 9 10
Comfortable Extreme discomfort

2. Rate the difficulty you experience in speaking due to dryness of your mouth and tongue:

0 1 2 3 4 5 6 7 8 9 10
Easy Extremely Difficult

3. Rate the difficulty you experience in chewing food due to dryness:

0 1 2 3 4 5 6 7 8 9 10
Easy Extremely Difficult

4. Rate the difficulty you experience in swallowing food due to dryness:

0 1 2 3 4 5 6 7 8 9 10
Easy Extremely Difficult

5. Rate the dryness your mouth feels when eating a meal:

0 1 2 3 4 5 6 7 8 9 10
No Dryness Extremely Dryness

6. Rate the dryness in your mouth while <u>not</u> eating or chewing:

0 1 2 3 4 5 6 7 8 9 10
No Dryness Extremely Dryness

7. Rate the frequency of sipping liquids to aid in swallowing food:

0 1 2 3 4 5 6 7 8 9 10
None required Extremely Frequent

8. Rate the frequency of fluid intake required for oral comfort when not eating:

0 1 2 3 4 5 6 7 8 9 10
None required Extremely Frequent

9. Rate the frequency of sleeping problems due to dryness:

0 1 2 3 4 5 6 7 8 9 10
None Extremely Frequent

10. Does your mouth feel dry when eating a meal? Yes / No

11. Are you thirsty? Yes / No

12. Does the amount of saliva in your mouth seem to be:

    _____ Too little
    _____ Too much
    _____ Don't notice it

13. Do you have difficulties swallowing any food? Yes / No

14. Do you sip liquids to aid in swallowing dry food? Yes / No

15. Have you smoked in the last week? Yes / No

    If yes, how many packs? _____

16. Do you drink alcohol more than twice a week? Yes / No

17. Do you have any medical problem/disease for which you take medication? Yes /No

    Which pills/medication do you take?

_____
_____
_____

INSTRUCTIONS: This survey is designed to assess how much you are bothered by your Head and Neck condition and/or treatment. Please answer every question by marking one box. If you are unsure about how to answer, please give the best answer you can.

1. As a result of your head and neck condition or treatment, over the past FOUR WEEKS how much have you been BOTHERED by your…

	Not at all	Slightly	Moderately	A lot	Extremely
A. Ability to talk to other people	☐	☐	☐	☐	☐
B. Ability to talk on the phone	☐	☐	☐	☐	☐

2. As a result of your head and neck condition or treatment, over the past FOUR WEEKS how much have you been BOTHERED by problems with…

	Not at all	Slightly	Moderately	A lot	Extremely
A. Volume of your voice	☐	☐	☐	☐	☐
B. Clarity of your voice	☐	☐	☐	☐	☐
C. Difficulty opening your mouth	☐	☐	☐	☐	☐
D. Dryness in your mouth while eating	☐	☐	☐	☐	☐

2 (continued). As a result of your head and neck condition or treatment, over the past FOUR WEEKS how much have you been BOTHERED by problems with…

	Not at all	Slightly	Moderately	A lot	Extremely
E. Chewing food (for example, pain, difficulty opening or closing your mouth moving food in your mouth, or teeth or denture problems)	☐	☐	☐	☐	☐
F. Swallowing liquids	☐	☐	☐	☐	☐
G. Swallowing soft foods and/or solids	☐	☐	☐	☐	☐
H. Your ability to taste food (For example, loss of taste, and/or loss of appetite due to poor taste)	☐	☐	☐	☐	☐
I. Pain, burning, and/or discomfort in your mouth, jaw, or throat	☐	☐	☐	☐	☐
J. Shoulder or neck pain	☐	☐	☐	☐	☐

3. Over the past FOUR WEEKS, how often did you take pain medication?...

Never	Rarely	Sometimes	Frequently	Always
☐	☐	☐	☐	☐

4. Over the past FOUR WEEKS how much have you been bothered by…

	Not at all	Slightly	Moderately	A lot	Extremely
A. Concerns or worries about Your appearance related to your head and neck condition or treatment	☐	☐	☐	☐	☐
B. Emotional problems related to your head and neck condition or treatment	☐	☐	☐	☐	☐
C. Embarrassment about your symptoms	☐	☐	☐	☐	☐
D. Frustration about your condition	☐	☐	☐	☐	☐
E. Financial worries due to medical problems	☐	☐	☐	☐	☐
F. Worries that your condition will get worse	☐	☐	☐	☐	☐
G. Physical problems related to your head and neck condition	☐	☐	☐	☐	☐

5. Were you working (employed) prior to being diagnosed with cancer? Yes ☐ No ☐ If no, got to question 6 (next page) →

5A. If yes, did your doctor declare you unable to work due to your head and neck condition or treatment? Yes ☐ No ☐

6. Have there been other problems related to your head and neck condition that were not mentioned? If so, please write them in the space below and tell us how much this problem has bothered you. (For instance, if your treatment included surgical transfer of tissue from a donor site to the head and neck, does the donor site bother you)

	Not at all	Slightly	Moderately	A lot	Extremely
A. _____	☐	☐	☐	☐	☐
B. _____	☐	☐	☐	☐	☐
C. _____	☐	☐	☐	☐	☐
7. For the past FOUR WEEKS, please rate your OVERALL amount of disturbance or BOTHER as a result of your head And neck cancer condition?	☐	☐	☐	☐	☐
8. Overall how satisfied are you with your Head and Neck cancer care at this Hospital?	☐	☐	☐	☐	☐

9. Overall how would you rate your response to treatment?

	Poor	Fair	Good	Very Good	Excellent
	☐	☐	☐	☐	☐

10. Approximately how long did it take you to answer this questionnaire? _____ Minutes

	Not at all	Slightly	Moderately	A lot	Extremely
11. How difficult was it to complete this questionnaire?	☐	☐	☐	☐	☐