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Cetuximab activity in dysplastic lesions of the upper aerodigestive tract ☆

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SUMMARY

Background: High risk head and neck mucosal premalignancy has a malignant conversion rate of up to 40%, despite adequate surgical therapy. Epidermal Growth Factor Receptor (EGFR) blocking agents, including cetuximab, have shown activity in head and neck squamous cell carcinoma (HNSCC) and have potential for therapy in high risk premalignancy.
Methods: We conducted a randomized, prospective, phase II clinical trial to determine the effects of cetuximab on patients with high risk premalignancy. Patients were randomized to treatment with cetuximab 400 mg/m^2 on week one followed by 250 mg/m^2 on week 2–8 or observation, with the option for crossover to cetuximab therapy for patients originally randomized to the observation arm.
Results: Two of 19 enrolled patients did not complete therapy due to treatment toxicity. Analysis of 17 patients who completed the trial regimen show a trend toward a larger mean decrease in grade of dysplasia in the cetuximab treated group (−1.0) vs. the observation group (−0.2) ($P = 0.082$, one-sided exact Wilcoxon rank sum test). However, in the observation group, none of the 5 patients (0%) achieved complete resolution of dysplasia; while 4 of 12 (33.3%) cetuximab treated patients had no remaining dysplasia after therapy.
Conclusions: Treatment of high risk premalignancy of the upper aerodigestive tract with cetuximab alone may result in significant, durable, and complete clinical and histological resolution of moderate to severe dysplasia in at least a subset of high risk patients. These results warrant further investigation in larger studies with increased statistical power.

Background

Over 45,000 new cases of HNSCC are diagnosed in the United States yearly, and this disease affects over 600,000 people worldwide [12]. Despite high cure rates for early stage cancers, the majority of late stage malignancies are fatal. Molecular genetic progression models and data on pre-malignant lesions have demonstrated that chromosomal loss occurs early in HNSCC progression. Early detection can improve patient survival and diminish the morbidity of treatment for advanced disease and hence

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improved methods of early detection and diagnosis has been actively pursued (see Figs. 1–5).

While premalignant lesions of the upper aero digestive tract may often be surgically removed, a proportion of patients with extensive premalignant disease cannot be treated with standard surgical excision. These patients are characterized by a clinical presentation of widespread dysplastic mucosa that extends beyond the boundaries of acceptable surgical resection. Other patients are characterized by a grossly normal appearing mucosa with histologic appearance of high-grade dysplasia that likewise cannot be adequately resected surgically and others recur despite surgical excision (see Tables 1 and 4).

Increased risk of progression to malignancy is also associated with prior head and neck cancer, advanced histologic grade, and evidence of genetic instability including chromosomal (allelic) loss. Those patients with moderate or severe dysplasia were noted to have a risk of progression to malignancy of approximately 60%, in a study with median 7 year follow up. Similarly, a prior history of head and neck cancer resulted in a 60% risk of progression in the same study [1]. Approximately 40% of patients with mild dysplasia or hyperplasia combined with 3p or 9p loss of heterozygosity (LOH) demonstrated progression to malignancy within 5 years [2,15].

Due to the challenging nature of surgical treatment for these patients, medical therapies are also attempted for patients with high-risk premalignant disease. Initial studies examining chemo preventive strategies for premalignant oral cavity disease were promising. These strategies were based on prior studies that used retinoids to decrease the incidence of all second primary tumors in head and neck cancer patients. Hong et al. demonstrated that high-dose 13 cis-retinoic acid (50–100 mg/m^2) prevented the development of second primary tumors in treated head and neck cancer patients [3]. On additional follow-up, patients who received 1 year of high-dose agent did not develop smoking-related malignancies *versus* those taking placebo. However, two years after completion of therapy, rates of second primary tumor development were identical in treated and placebo groups [4]. In addition, patients treated with high-dose 13-cis retinoic acid experienced substantial toxic effects, with the majority of patients requiring a dose reduction due to cheilitis, conjunctivitis, skin toxicities or effects on liver function. To date, however, additional, larger prospective trials examining retinoids and other agents have not consistently demonstrated efficacy for any agents in altering the natural course of premalignant oral disease and progression to malignancy, or were challenging to administer on a chronic, long-term basis [5–7].

Activation of the EGFR axis is well documented in oral premalignancy and analysis of prior retinoid trials demonstrate increased risk of malignant progression in patients with EGFR amplification [8,9]. In a subset analysis from a retinoid oral premalignancy trial, investigators found that an increased EGFR gene copy number is common in and associated with oral cancer development in patients with premalignant lesions expressing high EGFR levels. These data raise the possibility that a subset of oral premalignant lesions may benefit from specific therapeutic targeting of the EGFR axis [8,10].

As a result there has been significant interest in targeting the EGFR axis for chemoprevention or as therapy for high risk premalignancy. Investigators have completed a phase I trial that demonstrates favorable response rates for an oral EGFR tyrosine kinase inhibitor (TKI) and celecoxib, a cyclooxygenase 2 inhibitor demonstrating a synergistic effect with erlotinib in preclinical studies [11]. A phase III trial of Erlotinib (http://clinicaltrials.gov/show/NCT00402779) is the only phase III trial testing the activity of an EGFR directed agent in patients with leukoplakia at risk for oral cancer development.

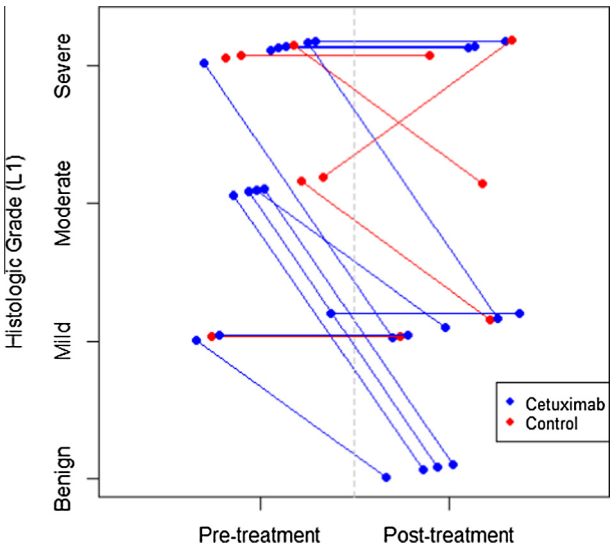

Fig. 1. Scatter plot of pre and post change in histology across treatment groups.

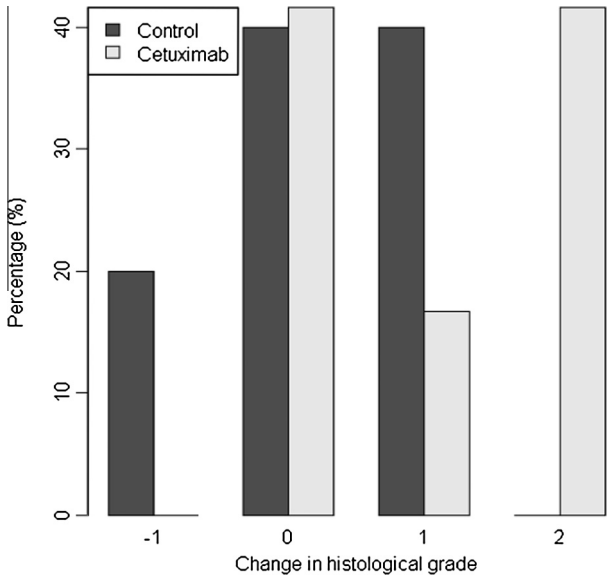

Fig. 2. Change in histological grade by treatment group.

To evaluate the potential for EGFR blockade to prevent premalignant progression in the upper aerodigestive tract, we conducted a phase II, randomized trial using cetuximab alone in the treatment of high-risk premalignant lesions of the head and neck. Patients were evaluated in terms of change in grade of dysplasia as a primary endpoint, with secondary endpoints to evaluate a clinical and histologic response, as well as changes in EGFR axis signalling.

Methods

Trial Design: This clinical trial was approved by the Johns Hopkins Medical Institutions review board (Clinicaltrials.gov identifier NCT00894413), and all human participants gave written informed consent to participate in the trial. This study was a prospective, two-arm, randomized, phase II trial of cetuximab treatment for patients with high-risk, premalignant UAD lesions. The control arm and treatment arm were randomized in a 3:1 ratio to increase the numbers of treated patients and thus maximize the possibility of statistically discerning low rates of response in the treatment

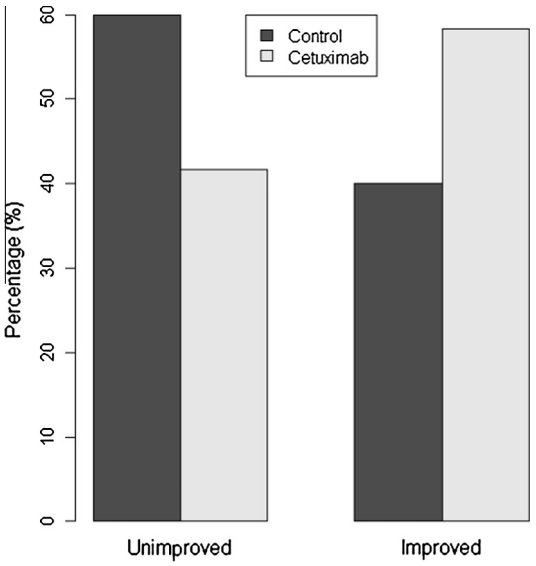

Fig. 3. Histological improvement by treatment group.

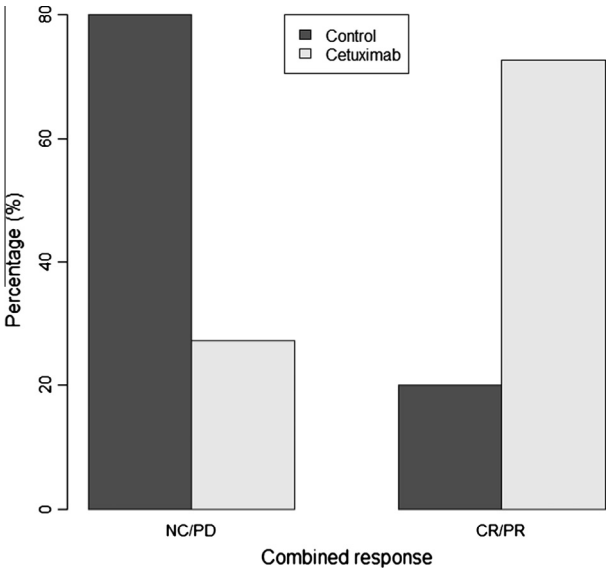

Fig. 5. Combined response by treatment group. *Percentage on *y*-axis represents proportion of subjects.

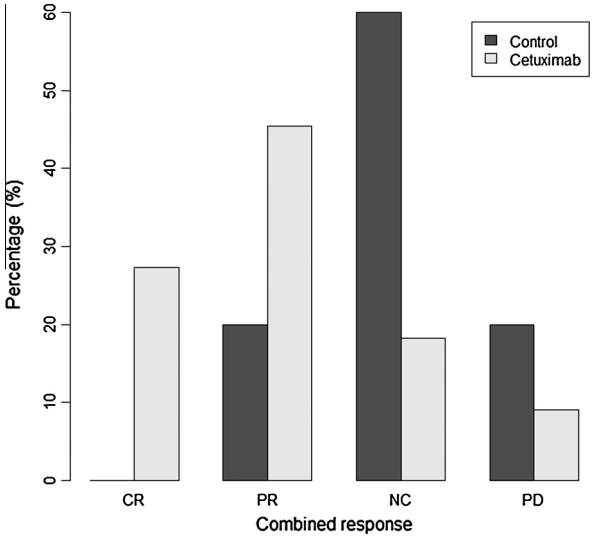

Fig. 4. Combined response by treatment group. *Percentage on *y*-axis represents proportion of subjects.

arm. The control arm was necessary to assess variability due to sampling and to variation in measurement of endpoints, although biologic changes were not expected over an eight-week period in untreated patients. Inclusion criteria included patients with premalignancy of the oral cavity, pharynx, or larynx with; (1) presence of 3p, 9p21, or 17p LOH, and/or (2) surgically unresectable high grade premalignant lesions, and/or (3) high grade premalignancy after curative therapy for HNSCC. Patients receive cetuximab 400 mg/m^2 on week one followed by 250 mg/m^2 on weeks 2–8. Patients in the control arm had the option of crossover to a treatment arm after completion of initial treatment. The *primary outcome* of the study was an objective response based on histologic grade with a *secondary outcome* based on clinical assessment that includes direct visualization of the lesion combined with histologic grade. Exploratory correlative studies evaluated the status of EGF-R pathway components and molecular alterations in pre- and post-treatment biopsies. Following the eight-week treatment with cetuximab, patients with resectable lesions were advised to undergo lesion resection based on the extent of initial disease. The primary objective of this phase II study was to determine the histologic response rate of high-risk UAD pre-malignant lesions to treatment with cetuximab.

Statistical considerations: Patients were randomized to treatment with cetuximab or control arm using a 3:1 allocation ratio. The sample size was determined by the fraction of subjects in each group whose histologic stage improves. A sample size of 60 (45 treatment: 15 control) provides 80% power for regression rates of 25% and 3% in treatment and placebo groups, respectively, one-sided alpha = 10%. The larger than usual type I error threshold was used because this trial was designed to be a precursor to a larger trial with longer-term clinical endpoints, and a difference of sufficient magnitude to produce a $P < 0.10$ would be suggestive enough to justify a larger RCT.

The primary analysis was based on the measure of histologic grade, which has 5 potential values, per WHO classification, and was considered as an ordinal variable:

1. benign hyperplasia
2. mild dysplasia
3. moderate dysplasia
4. severe dysplasia/carcinoma in situ
5. invasive cancer

For the purpose of the study, high-grade dysplasia was defined as moderate dysplasia, severe dysplasia, or carcinoma in situ. The histologic grade of each lesion was determined by at least two pathologists masked to the treatment group, and to other results in the same patient and the other pathologist. Consensus on grade was achieved by the study pathologists and reported as a single value. The within-person change in stage from pre to post treatment period was calculated using the above numerical scales and a trend difference was evaluated between treatment and control arms via one-sided exact Cochran–Armitage test and exact Wilcoxon rank sum test. In addition to access the treatment effect, risk factor analyses for the trend in histologic grade based improvement were evaluated via univariate logistic regression models where histological improvement was defined as improved if the histology score was at least 1 point lower in the post-treatment specimen compared to baseline, and as unimproved if the score was higher in the post-treatment specimen or was the

Table 1
Baseline characteristics of the study subjects.

Characteristics	Total (n = 19)	Control (n = 6)	Cetuximab (n = 13)
Gender			
Female	7 (36.8)	1 (16.7)	6 (46.2)
Male	12 (63.2)	5 (83.3)	7 (53.8)
Race			
White	18 (94.7)	6 (100)	12 (92.3)
Black	1 (5.3)	0 (0)	1 (7.7)
Smoking			
No	6 (31.6)	2 (33.3)	4 (30.8)
Yes	13 (68.4)	4 (66.7)	9 (69.2)
Alcohol			
No	6 (31.6)	2 (33.3)	4 (30.8)
Yes	13 (68.4)	4 (66.7)	9 (69.2)
Current drinker			
No	6 (31.6)	3 (50.0)	3 (23.1)
Yes	11 (57.9)	3 (50.0)	8 (61.5)
Unknown	2 (10.5)	0 (0)	2 (15.4)
Dysplasia site			
Oral cavity	13 (68.4)	3 (50.0)	10 (76.9)
Larynx	6 (31.6)	3 (50)	3 (23.1)
History of H&N cancer			
No	8 (42.1)	3 (50.0)	5 (38.5)
Yes	11 (57.9)	3 (50.0)	8 (61.5)
T Stage			
1	5 (45.5)	2 (18.2)	3 (27.2)
3	2 (18.2)	1 (9.1)	1 (9.1)
4	1 (9.1)	0 (0)	1 (9.1)
Unknown	3 (27.3)	1 (9.1)	2 (18.2)
N Stage			
0	3 (15.7)	1 (5.3)	2 (10.5)
1	3 (15.8)	1 (16.7)	2 (15.4)
2	2 (10.5)	0 (0)	2 (15.4)
Unknown	3 (15.8)	1 (5.3)	2 (10.5)
Prior surgery			
No	8 (42.1)	2 (10.5)	6 (31.6)
Yes	11 (57.9)	4 (66.7)	7 (53.8)
Unknown	0	0	0
Prior Radiation			
No	12 (68.4)	4 (21.1)	8 (42.1)
Yes	7 (36.8)	2 (33.3)	5 (38.5)
Unknown	0	0	0
Prior chemotherapy			
No	15 (78.9)	5 (26.3)	10 (52.6)
Yes	4 (21.1)	1 (16.7)	3 (23.1)
Unknown	0	0	0
Primary surgery			
No	9 (47.4)	3 (15.8)	6 (31.6)
Yes	10 (52.6)	3 (50.0)	7 (53.8)
Unknown	0	0	0
Primary radiation therapy			
No	16 (84.2)	6 (31.6)	10 (52.6)
Yes	3 (15.8)	0 (0)	3 (23.1)
Unknown	0	0	0
Primary chemotherapy			
No	17 (89.5)	6 (31.6)	11 (57.9)
Yes	2 (10.5)	0 (0)	2 (15.4)
Unknown	0	0	0
Post surgery chemotherapy			
No	17 (89.5)	5 (26.3)	12 (63.2)
Yes	2 (10.5)	1 (16.7)	1 (7.7)
Unknown	0	0	0
Post surgery radiation			
No	15 (78.9)	4 (21.1)	11 (57.9)
Yes	4 (21.1)	2 (33.3)	2 (15.4)
Unknown	0	0	0

same in the two specimens. Ordinal logistic regression models were also performed, treating changes in histological grade as an ordinal dependent variable. The risk factors included: patient gender, smoke status, current smoke status, alcohol use, current drinker, dysplastic sites, history of HN, prior surgery, prior radiation, prior chemo therapy, primary treatment of surgery, and primary treatment of radiation.

Secondary clinical endpoints were determined using both histology and clinical combined assessment. This endpoint was analyzed in a similar fashion as described above, where values were assigned to each response category. Study specific criteria were used for assessment of response which is described in supplementary material.

Results

25 patients were initially screened in the study, 6 were excluded at the time of screening due to the absence of high grade dysplasia after biopsy along with absence of 3p, 9p21, or 17p LOH (n = 4), pathologic diagnoses of invasive cancer at the time of screening (n = 2). Of the 19 patients that were screened positive and eligible to be randomized to one of the treatment arms, two of 19 enrolled patients did not complete therapy due to treatment toxicity. Of the 17 patients, 12 were in the treatment (cetuximab) arm and 5 in the observation group.

Descriptions of histologic staining and LOH are included in supplementary materials.

Of the 19 patients enrolled, 7 were female, 12 were male, 18 enrolled patients were Caucasian and one was Black. Inclusion criteria included patients with premalignant lesions of the oral cavity, pharynx, or larynx with; (1) presence of 3p, 9p21, or 17p LOH, and/or (2) surgically unresectable high-grade premalignant lesions, and/or (3) high grade premalignancy after curative therapy for HNSCC. Table 7 illustrates the pretreatment and post treatment histologies and LOH status.

Although the initial enrollment target was 60 patients across multiple sites, the enrollment could not be completed within the planned study duration due to low number of eligible patients presenting for treatment who qualified within the inclusion criteria resulting in a slower than anticipated accrual. Study enrollment could not be extended due to lack of additional funding to continue.

Two of 19 enrolled patients did not complete therapy due to treatment toxicity. Analysis of 17 patients who completed the trial show a trend toward a larger mean decrease in grade of dysplasia in the cetuximab treated group (−1.0) vs. the observation group (−0.2) (P = 0.082, exact one-sided Wilcoxon rank sum test). Meanwhile, there was an increasing trend in the proportion favoring improvement in histological grade with the cetuximab treatment, albeit not statistically significant (P = 0.103, exact Cochran–Armitage trend test). However, in the observation group, none of the 5 patients (0%) underwent complete resolution of dysplasia; while 4 of 12 (33.3%) cetuximab treated patients had no remaining dysplasia after therapy. In addition, three of these patients had complete resolution of dysplastic changes after cetuximab therapy alone without surgical excision. Of these three patients, two have had no recurrence of leukoplakia or dysplasia as of last follow up visit (1.5 and 2 years). The third patient treated for oral cavity dysplasia has had no recurrence of dysplasia or lesions within the oral cavity, but developed a hypopharyngeal cancer at 1.5 years after completion of cetuximab therapy. While the number of patients with NC/PD (No change/Progression of Disease) were similar in both groups, the number of patients with complete or partial response was significantly higher in the treatment group.

7 of 12 patients in the treatment group (58.3%) showed improvement in histologic grade, while only 2 of 5 patients in observation group (40%) showed any improvement resulting in a larger number of treated patients showed improvement in histologic grade (Table 2 and 3).

Table 2
Change in histological grade by treatment group.

Change in histological grade (pre–post)	*n* (%)	
	Control (*n* = 5)	Cetuximab (*n* = 12)
−1	1 (20.0)	0 (0)
0	2 (40.0)	5 (41.7)
1	2 (40.0)	2 (16.7)
2	0 (0)	5 (41.7)

Table 3
Association of histological improvement with treatment group.

Histological improvement	*n* (%)	
	Control (*n* = 5)	Cetuximab (*n* = 12)
Improved	2 (40.0)	7 (58.3)
Unimproved	3 (60.0)	5 (41.7)

Table 4
Univariate analysis of change in histology.

Characteristics	Change in histological grade[a]		Histological improvement[b]	
	OR (95% CI)[a]	*P*[a]	OR (95% CI)[b]	*P*[b]
Treatment				
Control	ref		ref	
Cetuximab	4.46 (0.63, 43.19)	0.152	2.10 (0.25, 21.01)	0.494
Gender				
Female	ref		ref	
Male	1.10 (0.18, 6.88)	0.915	0.75 (0.1, 5.29)	0.772
Smoking				
No	ref		ref	
Yes	2.06 (0.33, 14.75)	0.446	1.20 (0.16, 9.35)	0.858
Alcohol				
No	ref		ref	
Yes	2.89 (0.38, 28.54)	0.319	2.10 (0.25, 21.01)	0.494
Current drinker				
No	ref		ref	
Yes	6.12 (0.54, 156.07)	0.177	5.25 (0.48, 128.34)	0.207
Dysplasia site				
Oral cavity	ref		ref	
Larynx	0.94 (0.12, 7.31)	0.952	0.86 (0.08, 9.04)	0.893
History of H&N cancer				
No	ref		ref	
Yes	2.40 (0.38, 17.2)	0.358	3.50 (0.46, 35.01)	0.241
Prior surgery				
No	ref		ref	
Yes	0.24 (0.03, 1.75)	0.172	0.17 (0.01, 1.67)	0.165
Prior radiation				
No	ref		ref	
Yes	3.51 (0.48, 31.48)	0.230	6.25 (0.7, 83.07)	0.121
Prior chemotherapy				
No	ref		ref	
Yes	2.73 (0.26, 33.07)	0.400	4.50 (0.4, 111.1)	0.256
Primary surgery				
No	ref		ref	
Yes	0.42 (0.06, 2.78)	0.375	0.40 (0.04, 3.25)	0.403

OR = odds ratio.
[a] Includes categories of −1, 0, 1, and 2; *P* values obtained using ordinal logistic regression.
[b] Improved vs. unimproved; *P* values obtained using logistic regression.

9 of 11 (81.8%) patients in treatment group showed a combined response of clinical and histologic change compared to only 2 of 5 (40%) in the observation group (Table 5).

Univariate analysis of combined response was completed based on treatment status, gender, smoking and alcohol status, site of dysplasia, history of prior head and neck cancer and primary and prior treatments (Table 6). While not statistically significant, the treatment group did show a higher rate of combined response. Additionally, patients with a prior history of head and neck cancer had a higher combined response rate, albeit not statistically significant.

Pre and Post treatment LOH was performed on 11 of the 19 patients, of these 8 were in the treatment group while 3 were in the observation group. 5 of the 8 patients in the treatment group showed a change in LOH after treatment (62.5%) while none of the 3 patients in the control group showed any change ($P = 0.182$, Fisher's exact test). Change in LOH after treatment was observed in patients treated with CTX (62.5%), although not statistically significant ($P = 0.062$, exact McNemar's test comparing pre and post difference). 4 of the 5 patients who a change in LOH had a positive correlation with combined response, while the 5th patient had a change in LOH but no change in combined response. None of the observation group patients showed post treatment change in LOH status (Table 7).

Of the 5 patients randomized to observation, three elected to crossover to cetuximab therapy, and two of these three patients had complete resolution of dysplasia following cetuximab treatment.

Development of rash has been associated with response to treatment with cetuximab in head and neck cancer patients. On the current trial, 12 of the 19 patients were randomized to treatment arm and subsequently were treated with cetuximab. 6 of the 12 patients treated with cetuximab developed a rash, 4 of whom had a response to treatment and had no remaining dysplasia after treatment. Of the 6 patients on the treatment arm who did not develop a rash, 3 had response to treatment showing improvement in histologic grading. Additionally, one patient on the observation arm, who crossed over to treatment arm and was treated with cetuximab developed rash and responded to treatment with no dysplasia after treatment.

Limited data were available on the correlation with EGFR axis alterations with therapy and clinical response due to limitations on remaining material to perform immunohistochemical assays, however, these data are included in supplementary material.

Discussion

Current approaches to predicting development of cancer in patients with pre-malignant lesions and in patients with prior cancers combine histologic and molecular techniques. Chromosomal changes are particularly useful for prediction, especially given the observation that histologically normal appearing mucosa often harbor HNSCC-associated genetic changes. Many patients with premalignant dysplastic lesions cannot be adequately treated with surgical excision. Many patients present with relatively widespread dysplastic involvement of the mucosa that extends beyond the boundaries of acceptable surgical resection. Other patients are characterized by a grossly normal appearing mucosa with the histologic appearance of high-grade dysplasia that likewise cannot be adequately resected surgically. Furthermore, genetic molecular changes may be present in tissues with mild dysplasia or benign histologic findings but are at risk of progression to cancer. Increased risk of progression to malignancy is also associated with prior head and neck cancer, advanced histologic grade, and evidence of genetic instability including chromosomal loss.

The low number of enrolled patients may have resulted in the lack of statistically significant change in histologic, clinical and combined responses in treated patients when compared to

Table 5
Combined response by treatment group.

Combined response	*n* (%)	
	Control (*n* = 5)	Cetuximab (*n* = 11)
CR	0 (00.0)	3 (27.3)
PR	1 (20.0)	5 (45.4)
NC	3 (60.0)	2 (18.2)
PD	1 (20.0)	1 (9.1)

Table 6
Univariate analysis of combined response.

Characteristics	Ordinal response[a]		Binary response[b]	
	OR (95%CI)[a]	*P*[a]	OR (95%CI)[b]	*P*[b]
Treatment		0.066		0.0701
Control	ref		ref	
Cetuximab	7.50 (0.98,81.17)		10.67 (1.06,262.56)	
Gender		0.954		0.288
Female	ref		ref	
Male	0.95 (0.15,6.03)		0.32 (0.03,2.43)	
Smoking		0.855		0.518
No	ref		ref	
Yes	1.20 (0.17,8.55)		0.50 (0.05,3.92)	
Alcohol		>0.999		0.839
No	ref		ref	
Yes	1.00 (0.14,7.39)		0.80 (0.08,6.89)	
Current drinker		0.750		0.7334
No	ref		ref	
Yes	1.43 (0.15,13.86)		1.50 (0.13,17.42)	
Dysplasia site		0.945		0.389
Oral cavity	ref		ref	
Larynx	0.92 (0.09,10.84)		0.31 (0.01,4.12)	
History of H&N cancer		0.157		0.070
No	ref		ref	
Yes	4.20 (0.61,35.55)		10.67 (1.06,262.56)	
Prior surgery		0.350		0.872
No	ref		ref	
Yes	0.38 (0.04,2.81)		0.83 (0.08,7.78)	
Prior radiation		0.315		0.181
No	ref		ref	
Yes	2.87 (0.38,25.69)		5.00 (0.53,68.31)	
Prior chemotherapy		0.670		0.322
No	ref		ref	
Yes	1.61 (0.18,15.49)		3.75 (0.33,93.86)	
Primary surgery		0.626		0.641
No	ref		ref	
Yes	0.62 (0.08,4.25)		1.67 (0.19,15.67)	

OR = odds ratio.
[a] Combined response coded as an ordinal variable (CR, PR, NC and PD), and odds ratios and *p* values obtained using ordinal logistic regression.
[b] Combined response coded as an binary variable (CR/PR vs. NC/PD), and odds ratios and *p* values obtained using simple logistic regression.

patients in the observation group. However, a larger percentage of treated patients presented with a histologic downgrade when compared to observed patients. This supports the theory that targeting the EGFR for prevention or treatment of high risk premalignant lesions is a potential option for patients who lack other treatment options, since 72.7% of the treated patients showed a combined response. Despite the fact that a small number of patients in the treatment arm had progression of disease, the results provide evidence that cetuximab shows potential in a chemoprevention strategy. Of note, 3 patients have lasting complete responses at 2 year follow up visits.

LOH profiles have been shown to predict risk of progression of disease in patients with high risk premalignant lesions. Despite concerns with low number of enrolled patients and toxicity concerns in a subset of patients, the trial showed a trend toward an association with LOH data (Table 7).

Although the EPOC trial [14], although failed to reduce oral cancer free survival in high risk patients, the preliminary reports show LOH status as a marker for risk. Patients with histological evidence of an oral premalignant lesion (with or without a prior history of invasive OC) underwent LOH profiling, of 375 patients evaluated for LOH status, 254 were LOH positive, and 121 were LOH negative. LOH negative patients received no treatment. LOH positive patients were randomized (1:1) to erlotinib 150 mg per oral daily for 12 months or placebo. The EPOC trial also showed positive response in mucosal lesions at 1 year following treatment although the primary endpoint was progression to cancer. The results showed a larger number of LOH positive patients progressing to oral cancer, as compared to LOH negative patients. In our trial, despite the low number of subjects, a significant number of patients showed a change in LOH status after treatment which correlated with the combined response.

In a similar study targeting the EGFR [13], using Celecoxib and Erlotinib, the results showed a clinical response that correlated with the down regulation of the activated protein levels of the EGFR pathway.

The most definitive trial to date (EPOC) examined the utility of Erlotinib, an EGFR tyrosine kinase inhibitor, in the prevention of progression and secondary oral cancer in a high risk population. In this trial, LOH was validated as a marker of oral cancer risk and found to be associated with increased *EGFR* copy number. Erlotinib did not, however, improve CFS in high-risk patients with LOH-positive or high-*EGFR*-gene-copy-number OPLs. This trial supports incorporation of LOH testing as a prognostic tool in routine clinical practice but does not support erlotinib use in this setting.

Considering results from the EPOC trial and another prospective study [15] which showed LOH status predicts progression to invasive disease and our study showing cetuximab in selected patients reverted the LOH status to negative, these results support the interest in the potential use of cetuximab for patients who are not good surgical candidates. Patients with prior head and neck cancer have a higher risk of progression of disease, this group of patients also show a significantly higher combined response to treatment with cetuximab, providing a treatment strategy which would both treat the dysplasia while at the same time reduce the risk of progression of disease. Of note, Cetuximab has been shown to be active in combination with radiotherapy for invasive head and neck cancers. Recent data indicate that Cetuximab may exert its effect via a combined activity of inducing immunologic response to head and neck cancers as well as direct effects on the EGFR signal transduction axis. It is possible that the responses seen in premalignancy in this study may also have an immune component as well.

Due to the challenging nature of surgical treatment for these patients, including size and location of lesions, potential recurrence following attempted excision, regional extent of molecular change and the lack of clinically available markers of molecular change, medical therapies may have utility in patients with high-risk premalignant disease. While this trial did not complete intended accrual, it did demonstrate that EGFR blockade with cetuximab

Table 7
LOH status and combined response.

Patient no.	Randomization	Histology – pre Rx/observation	Histology – post Rx/observation	Pre RX LOH	Post RX LOH
1	CTX	Mild dysplasia	Reactive change	+	–
2	CTX	Severe	Mild	+	–
3	Observation	Mild dysplasia	Mild dysplasia	+	+
4	CTX	Lesion 1 – mild dysplasia Lesion 2 – mild dysplasia	Lesion 1 – mild dysplasia Lesion 2 – no dysplasia/ulcerated	+	–
7	Observation	Severe	Severe	+	+
8	CTX	Moderate	Hyperplasia	+	–
9	CTX	Moderate	Mild	+	+
12	CTX	Severe	CA in situ	+	+
13	CTX	Severe	CA in situ with superficial invasion	+	+
15	Observation	Moderate	Mild	+	+
19	CTX	Mild	Mild	+	–

alone may result in significant, durable, and complete clinical and histological resolution of moderate to severe dysplasia in at least a subset of high risk patients. These results warrant further investigation in larger randomized controlled trials.

Conflict of interest statement

None declared.

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The manuscript/analysis of this article is based on a web database application provided by Research Information Technology Systems (RITS) – https://www.rits.onc.jhmi.edu/.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.oraloncology.2015.11.016.

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