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A Randomized Multi-Institutional Phase II Trial of Everolimus as Adjuvant Therapy in Patients with Locally Advanced Squamous Cell Cancer of the Head and Neck

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Abstract

Purpose: Investigate whether adjuvant everolimus, an mTOR inhibitor, improves progression-free survival (PFS) in advanced stage head and neck squamous cell carcinoma (HNSCC) and provide outcomes related to correlative biological factors associated with disease control.

Patients and Methods: This was a prospective, randomized, double-blind phase II trial of advanced stage HNSCC patients from 13 institutions who were confirmed disease-free post-definitive therapy and enrolled between December 2010 and March 2015. Patients received adjuvant everolimus or placebo daily (10mg, oral) for a maximum of 1 year. p16 IHC as a surrogate marker for HPV infection and whole exome sequencing were performed. Cox proportional hazard models estimated hazard rates. Log-rank tests evaluated differences in survival. The primary endpoint was PFS. Secondary endpoints and objectives included overall survival (OS) and toxicity assessment.

Results: 52 patients (median [range] age, 58, [37-76] years; 43 men [83%], 9 women [17%]) were randomized to placebo (n=24) or everolimus (n=28). PFS favored everolimus, but was not significant (log-rank P=0.093; HR=0.44, 95% CI: 0.17-1.17). There was no difference in OS (P=0.29; HR=0.57, 95% CI: 0.20-16.2). Everolimus resulted in significant improvement in PFS for p16-negative patients (n=31) (P=0.031; HR=0.26, 95% CI: 0.07-0.97), although subgroup analysis showed no difference for p16-positive patients (n=21) (P=0.93). Further, PFS was significantly higher in *TP53* mutated (*TP53mut*) patients treated with everolimus compared to placebo (Log-Rank P=0.027; HR=0.24, 95% CI: 0.06-0.95). No treatment difference was seen in patients with *TP53* wild-type (*TP53wt*) tumors (p=0.79).

Conclusions: p16-negative and *TP53mut* patients may benefit from adjuvant treatment with everolimus.

Trial Registration: ClinicalTrials.gov Identifier: NCT01111058

Statement of Translational Relevance:

Advanced stage head and neck squamous cell carcinoma (HNSCC) patients are at a high risk of recurrent disease. Due to dismal 5-year survival rates, such patients are in dire need of effective adjuvant therapy. Everolimus, an mTOR inhibitor, has documented activity in HNSCC and is well tolerated with minimal long-term toxicity. This placebo-controlled phase II trial is the first to show promising results using everolimus as adjuvant therapy after complete response to definitive treatment in a subset of HPV-negative patients with advanced stage disease. In particular, HPV-negative TP53 mutated tumors appear to yield the best benefit. Thus, subsequent trials using everolimus in this patient population are warranted.

Keywords

Everolimus; head and neck cancer; adjuvant therapy; advanced stage; mTOR inhibitor

Introduction

Approximately two-thirds of tobacco-related HNSCC patients present with local-regional advanced disease, frequently due to late diagnosis. The current approach for curative intent includes chemoradiotherapy or surgery plus adjuvant radiotherapy ±chemotherapy, with immunotherapy, targeted agents and chemotherapy reserved for recurrent disease.[1]

There are no adjuvant protocols to reduce the high risk of recurrence in HNSCC after definitive therapy as commonly used in colon and breast cancer.[2, 3] Efforts to improve outcome have been attempted, but have proven ineffective, resulted in unacceptable toxicity and/or adverse events, or both.[4] A recent meta-analysis of randomized trials from 1965-2016 robustly documented no survival benefit for adjuvant chemotherapy in non-metastatic HNSCC.[5] Hence, agents with low toxicity profiles, such as targeted agents, should be explored.

Many pathways altered by mutation/amplification in HNSCC converge on downstream activation of the Akt/mTOR pathway, making it a rational target for tertiary prevention.[6, 7] Expression of eukaryotic initiation factor 4E (eIF4E) is functionally active through activation of the Akt/mTOR signaling pathway; moreover, overexpression of eIF4E in histologically tumor-free surgical margins of HNSCC patients was an independent predictor of recurrence.[8, 9] An exploratory biomarker trial with temsirolimus, an mTOR inhibitor, in newly diagnosed advanced stage HNSCC patients showed inhibition of the Akt/mTOR pathway in tumors and PBMCs (surrogate markers) and proapoptotic activity of temsirolimus.[10] Similar results were noted in a window of opportunity trial with rapamycin, in which objective clinical responses were observed in 25% of the patients enrolled.[11] A phase I trial with everolimus, cisplatin and radiotherapy showed HNSCC patients tolerated everolimus at therapeutic doses (up to 10 mg/day) and that the regimen merits further evaluation, especially among patients who are status post resection harboring eIF4E in histologically negative surgical margins.[12] Given these preclinical and clinical data in HNSCC, the role of mTOR inhibitors as adjuvant therapy was explored.

Everolimus is an mTOR inhibitor that has been used as an immunosuppressant in solid organ transplantation since 1996 and more recently as an anti-cancer agent.[13-16] Everolimus is being investigated in cancers based on its potential to act directly on tumor cells by inhibiting cell proliferation, as well as indirectly by inhibiting angiogenesis leading to reduced tumor vascularity.[17] Oral formulations (5 mg, 10 mg) are approved for patients with advanced renal cell carcinoma.

The purpose of this trial was to assess whether adjuvant therapy with everolimus could significantly improve two-year and overall progression-free survival (PFS) in patients at high risk of cancer recurrence who were free of disease after definitive local therapy for advanced stage HNSCC.

Methods

Study Design and Objectives

The trial was coordinated and funded through the University of Chicago Personal Cancer Care Consortium. Novartis Pharmaceuticals sponsored site participation in this investigator-initiated trial (NCT01111058). This was an IRB approved multi-institutional randomized double-blind phase II clinical trial of everolimus (intervention) versus placebo. The study was compliant with Good Clinical Practice guidelines, US 21 Code of Federal Regulations, and the Declaration of Helsinki. Written informed consent was obtained by all participants. A summary of study design is presented in Figure 1.

Key eligibility criteria included TNM stage IVa or IVb (AJCC 6th edition at the time of enrollment; patients were later restaged and reported as AJCC 7th) HNSCC of the oral cavity, oropharynx, hypopharynx or larynx, Karnofsky performance status score >70%, adequate bone marrow, hepatic and renal function, and no evidence of disease within 16 weeks (to allow adequate time for PET confirmation and enrollment) after curative intent therapy. Oropharynx patients were required to be p16/HPV-negative or p16/HPV-positive with a minimum tobacco exposure history of 10 pack years. Exclusion criteria were patients who received anticancer therapies within 4 weeks of study drug initiation (including chemotherapy, radiation therapy, antibody-based therapy, etc.), systemic treatment with corticosteroids or immunosuppressors, acute radiotherapy related mucositis or dermatitis, metastatic disease, other malignancies in the past three years, previous treatment with mTOR inhibitors, or other severe medical conditions that could have affected participation.

Randomization was stratified for disease stage, local therapy type (IVa surgical vs. IVa non-surgical vs. IVb) and treating institution. Within 16 weeks after completion of definitive, curative-intent therapy for locally advanced HNSCC, patients received either 10 mg daily of oral everolimus or placebo for a maximum of 1 year. As PET is typically obtained at 12 weeks post-therapy to accurately determine complete response, the study allowed up to 16 weeks to account for any delay. Gastrostomy tube administration was allowed. The targeted sample size was 160 patients.

PET scans confirmed complete response to primary treatment prior to starting therapy and labs, clinical examination and/or scans were conducted at 4, 16, 32 and 52 weeks after initiating therapy. Patients were followed up for a minimum of 2 years. Disease progression was evaluated by clinical and radiographic methods and by clinical pathology if necessary. The primary endpoint was progression-free survival (PFS). Secondary endpoints and objectives included overall survival (OS), toxicity assessment and site of disease progression. We also wanted to determine whether genetic aberrations in PIK3/Akt/mTOR pathway are associated with recurrence rates and efficacy of everolimus. Next Generation Sequencing (NGS) was used to detect somatic mutations in primary pre-treatment tumor samples of patients to evaluate whether an association exists between cancer-associated mutations and favorable outcomes to mTOR-targeted therapy.

Procedures

Everolimus Administration—Everolimus and placebo were supplied by Novartis. Treatment compliance was maintained through individual drug diaries and drug accountability noted by the return of study medication. Dosing was self-administered orally as 10 mg (two 5 mg tablets) once daily from study day 1 until disease progression, unacceptable toxicity or once 1 year was reached. Instructions were given to take doses in the morning at the same time each day.

For patients unable to tolerate the dosing schedule or if unacceptable toxicity occurred as defined by grade, dose adjustments or interruptions were permitted. Patients were kept at the initial dose level (10 mg daily) when toxicity was tolerable. If dosing became intolerable to the patient or if grade-defined unacceptable toxicity occurred, everolimus was interrupted until recovery to grade ≤1, then reintroduced at the same or lower dose (5 mg daily or every other day), depending on event type and severity. Grade 4 events resulted in discontinuation. Toxicity was assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 4.0.

Patients whose treatment was interrupted or discontinued due to an adverse event suspected to be related to everolimus were followed at least weekly until the adverse event returned to grade 1. If a dose delay of > 21 days was required, treatment was discontinued.

Research Testing

Specimens—Tissue obtained prior to curative intent therapy from the diagnostic biopsy and/or during surgical resection, serum and whole blood samples were used for the correlative studies. Serum and whole blood were collected at baseline and at weeks 4, 16, 52, then stored at −80° C.

Immunohistochemistry—For patients with available tissue, $p16^{INK4a}$ immunohistochemistry was performed using a mouse monoclonal anti-p16 antibody clone E6H4 on the BenchMark platform (CINtec Histology, Ventana). Interpretation was performed by a certified pathologist and results given as either negative or positive.

Next-Generation Sequencing—Clinical grade targeted next generation sequencing was performed on HNSCC DNA from formalin-fixed paraffin embedded (FFPE) tumor sections of 44/52 patients. Potential germline variants were eliminated (dbSNP 141) unless they belonged to the catalog of somatic mutation in cancer (COSMIC v82) and were rare in the population (ExAC v0.3 MAF<1E-3). Mutations were then annotated using ref Gene and filtered for non-silent effects. Raw variants were further filtered and annotated using variant-tools. Under this framework, variants were annotated in regard to RefSeq reference annotation using ANNOVAR. Only non-silent variants, including variants in splice sites affecting the open reading frame of the genes were retained. Potential germline variants included matching variants in dbSNP (version 141) were eliminated unless they were also present in the catalog of somatic mutation in cancer (COSMIC v82). Next, variants present in the ExAC (v0.3) at a population minor allelic frequency of 0.001 or greater were further excluded. Furthermore, variants identified 7 or more tumors were eliminated after

confirming they did not correspond to known cancer mutational hotspots in oncogenes. Finally, the variants remaining and affecting known cancer genes from the Cancer Gene Census were manually curated by subject matter experts to eliminate rare unambiguous germline variants and alignment artifacts.

The mutations located in known HNSCC mutated genes were manually curated to eliminate technical artifacts. A total of 25 mutations in *TP53* were identified in 23 patients and were further used as biomarkers for the association, where only non-silent mutations were included.

Statistical Analysis

Participants were randomized to two arms: placebo and everolimus (intervention) using permuted blocks. Patient demographics and tumor characteristics were obtained. Continuous variables are presented as mean (SD or range), whereas categorical variables are presented as number (percentage) of patients. PFS and OS were estimated by the Kaplan-Meier (1958) method and Cox (1972) proportional hazard models estimated hazard ratios (HR). Log-rank tests evaluated differences in survival. PFS was defined as the time from randomization to disease progression or death from any cause; however, if death occurred over one year after the patient's last negative exam, the patient was censored as of the date last known progression free. Data analyses were performed with STATA statistical software, version 16 (Stata Corp).

Sample Size—The target sample size was 160 subjects (80 per arm). This sample size was chosen in order to provide 80% power at two-sided α=0.05 to detect a hazard ratio of 1.94, corresponding to a 70% vs. 50% difference in the PFS rate at two years under exponential survival distribution assumptions, using a log-rank test and allowing for a 25% loss to follow-up rate. It also assumed three years of accrual and two years of further follow-up. The HR was based on a 2-year PFS that would be clinically meaningful – 50% to 70%, the preclinical animal data, and the hazard ratio associated with activated mTOR in patient samples from previous studies.

Data Availability Statement

Sequencing data are available under NIH dbGaP Accession: *phs002986.v1.p1*. Additional study data are not publicly available due to information that could compromise patient privacy, but are available upon reasonable request from the corresponding author.

Results

The trial was terminated prior to achieving the accrual goal due to slow accrual rates. Of the 59 patients consented and screened, 7 were ineligible based on study criteria. A total of 52 patients from 13 institutions participated from 2010 to 2015 (mean age, 58 [range 37-76]) and randomized to receive either placebo (n=24) or everolimus (n=28). Distributions of baseline variables by treatment arm are detailed in Table 1.

There was equal distribution between all variables except T stage. Advanced T stage T4a and T4b were skewed towards the everolimus group (57%) v/s placebo group (33%).

With regard to nodal status and margins, 4 of the 25 patients who underwent surgery had extranodal extension (2 in each arm) while 4 had positive margins (1 in the placebo group and 3 in the everolimus arm). In terms of adjuvant treatment, 17 received both cisplatin plus RT (9 in the placebo arm and 8 in the everolimus group). Eight patients received either cisplatin or RT (1 placebo patient, 7 everolimus patients). In the intent-to-treat analysis, all 52 patients contributed data to the longitudinal analysis of survival. During and after the one year of assigned treatment both groups were followed for progression, during which these participants were considered to be at risk of tumor relapse.

PFS favored everolimus, but the difference was not statistically significant (2-year absolute difference 22.9% (95% CI −6.7 to 52.1%), P=0.13; log-rank test for equality of survival functions, P=0.093; HR=0.44, (95% CI: 0.17-1.17)). Eighteen patients experienced recurrence or died within one year of the last known progression-free date (6 everolimus; 12 placebo). Two patients died more than one year after the last known progression-free interval date, one in each arm, and were censored in PFS analysis. Adjusting for other treatments received (surgery, RT, induction chemotherapy) had little effect on the estimated everolimus effect (adjHRs of 0.45, 0.46, and 0.46, respectively). Adjusting for pack-years of smoking also did not materially alter the treatment effect estimate (adjHR=0.51, 95% CI: 0.12, 2.18), although this analysis included only 24 observations due to missing data. There was no significant difference in OS (log-rank P=0.29; HR=0.57, 95% CI: 0.20-16.2) (Figure 2). Adjusting for other treatments received yielded similar results (adjHR=0.55, 0.55, and 0.57 for surgery, RT, and induction chemotherapy, respectively. Adjusting for pack-years of smoking also gave similar results (adjHR=0.84, 95% CI: 0.21-3.39) among those with smoking history available.

As shown in Figure 3A, everolimus treatment was significantly associated with longer PFS for p16-negative patients (log-rank P=0.031; HR=0.26, 95% CI: 0.07-0.9) while no difference was observed in p16-positive patients (log-rank P=0.93; HR=0.93, 95% CI: 0.18-4.64), although the latter is based on few events.

Next Generation Sequencing

After extensive filtering to eliminate potential artifacts and likely germline variants (“Methods”), we identified a total of 796 non-silent somatic mutations (693 SNP, 103 indels) affecting 335 genes in 44 available tumors. *TP53* was the most frequently mutated gene, affecting 23/44 tumors. As expected, HPV-negative tumors were more likely to be *TP53mut* (21/25, 84% compared to 2/19, 11% in HPV-positive tumors, p<0.001). Subgroup analysis of *TP53* mutational status was associated with significantly higher PFS rates in *TP53mut* patients treated with everolimus compared to placebo (log-rank P=0.027; HR=0.24, 95% CI: 0.06-0.95). As a result of the small sample size, the type of *TP53mut* that benefitted from everolimus could not be deciphered. Remarkably, this difference between everolimus vs. placebo was not seen in the *TP53wt* group (P=0.79; HR=1.30, 95% CI: 0.18-9.29) as shown in Figure 3B, although limited by few events. No statistically significant difference was observed for OS in either *TP53wt* (P=0.69; HR=1.50, 95% CI: 0.20-11.1) or *TP53mut* (P=0.13; HR=0.30, 95% CI: 0.06-1.55) patients. Everolimus treatment did not significantly

affect OS in either p16-positive or p16-negative patients (log-rank P=0.82; HR=1.26, 95% CI: 0.17-9.50 and P=0.10; HR=0.34, 95% CI: 0.09-1.32 respectively).

TP53 Subset Analysis

A total of 25 mutations in *TP53* were identified in 23 patients and were further used as a biomarker for the association with endpoints. The type of mutations observed were: Nonsense (6), frameshift (5), splicing (1), missense (12) and inframe deletion (1) (Supplementary Table S1). In the placebo arm, 46% (n=11) of patients were *TP53wt*, 42% (n=10) were *TP53mut* and 12% (n=3) unknown. In the everolimus arm, 36% (n=10) were *TP53wt*, 46% (n=13) were *TP53mut* and 18% (n=5) unknown. We observed no significant difference in *TP53* mutational status between treatment groups (Pearson chi^2=0.35, P=0.56). To assess the possibility of *TP53* status being disproportionate with regards to initial standard of care therapies, we determined that the increase in PFS comparing everolimus to placebo in *TP53mut* patients occurred despite similar definitive treatment modalities (Supplementary Table S2).

Toxicity

Adverse events at least possibly related to study drug are summarized in Table 2 (full version Supplementary Table S3). Everolimus was generally well tolerated. Thirteen patients required a dose modification. Twelve patients (43%) experienced a grade 3 or higher adverse event attributed to study drug. The most frequent (>20%) adverse events from baseline were stomatitis/pharyngitis, decreased lymphocyte and platelet counts, anorexia and fatigue. A single grade 4 hyperbilirubinemia event resulted in discontinuation of everolimus for probable attribution to the drug in one patient. Two patients experienced serious adverse events possibly related to study drug: One patient was hospitalized for a skin infection and one patient experienced a thromboembolic event.

DISCUSSION

Many targeted agents in the adjuvant setting after definitive therapy have been tested in HNSCC, but have failed. Afatinib, an ERBB blocker, has shown efficacy in both recurrent and metastatic HNSCC.[18] However, treatment with Afatinib after CRT did not improve disease-free survival and was associated with more adverse events than placebo in patients with primary, unresected, clinically high- to intermediate-risk HNSCC.[19] Anti-EGFR targeted therapies initially held high expectations, but the only FDA-approved targeted agent for HNSCC, Cetuximab, has shown a poor response rate and likely intrinsic resistance.[20, 21] The EGFR/Erb2 inhibitor lapatinib proved unsuccessful as concomitant treatment, followed by 12 month maintenance therapy.[22] Trials exploring immunotherapy as adjuvant therapy and in combination with RT or CRT after surgery are ongoing[23, 24] however, recent data have been negative[25].

Although HPV-associated HNSCC are the most rapidly growing tumors in the USA, worldwide smoking is still the leading cause of HNSCC. HPV-positive oropharynx patients with a >10 pack year smoking history and advanced stage HPV-negative patients have a 5-year survival of 65% and <30%, respectively.[26, 27] Approximately 80% of HPV-negative

tumors have *TP53* mutations and this group of patients has the worst prognosis as noted in both the RTOG 0522 and ECOG 2399 trials.[21, 28] Our dataset showed similar results where 84% (21/25) of p16-negative patients in our cohort had *TP53mut* compared to 11% (2/19) in p16-positive patients. One limitation of the trial was the later downstaging of HPV/p16+ patients in the updated AJCC 8th edition. As study design predated current intermediate risk groups, Ang et al's NEJM report describing HPV+ patients with >10 pack years was used to make this determination.[26] Interestingly, although the study enrolled a final small sample size of 52 patients, everolimus showed statistically significant improvement in PFS among p16-negative (58%) patients, with no benefit over placebo for p16-positive (42%) tumors. Given the small number of p16-positive patients and that they have a significantly better disease-free survival, the difference between everolimus and placebo may not have been seen. However, these patients do well and may not benefit from adjuvant therapy.

Furthermore, the patient subset whose tumors harbored a *TP53* mutation (52%) demonstrated a significant difference in PFS. *TP53* is the most frequently mutated gene in HNSCC, whose alterations significantly affect tumor progression and resistance to treatment.[29-31] The Cancer Genome Atlas consortium showed that *TP53mut* HNSCC have worse prognosis and survival outcomes compared to *TP53wt* tumors.[32] Given the PFS benefit observed with everolimus based on *TP53* status, we then analyzed the treatment modalities received in both the *TP53mut* and *TP53wt* group and found no significant differences.

The reason for the enhanced clinical benefit of everolimus in *TP53mut* patients is at the present not fully understood. In general, a consistent biomarker in HNSCC associated with prognosis is p16, a surrogate for HPV infection.[26, 33] As such, most p16-positive tumors are *TP53wt*, consistent with the low frequency of *TP53mut* in HPV+ HNSCC lesions, even when only p16-positive patients with a 10-pack year smoking history were enrolled.[34-36] As HPV-positive patients exhibit a better prognosis, [37] which we also observed, everolimus may not be able to display further clinical benefit in this group. Alternatively, mutations in *TP53* may sensitize HNSCC to mTOR inhibition. Preliminary studies from our lab have shown that mTOR inhibitors induced autophagy dependent cell death in HPV-negative *TP53mut* HNSCC cell lines, providing a novel mechanism of action.[38] Recent studies have revealed that WT p53 regulates protein translation through the induction of 4E-BP1.[39] Hence, one possibility is that *TP53mut* HNSCC lesions may have lost this translational control mechanism and therefore become vulnerable to the tumor suppressive effect of 4E-BP1 when unleashed downstream from mTOR inhibition.[40] These, and other possibilities are under current investigation. As *TP53mut* patients are noted in 80% of HPV-negative tumors and have extremely poor outcomes, everolimus holds significant promise in this group of patients who are arguably most in need of more effective options.

Patients received everolimus or placebo for one year. Future directions for everolimus as adjuvant therapy should consider extending the length of time on drug, as it was very well tolerated in our study and prolonged use is standard of care in renal cell carcinoma; [41, 42] or possibly in combination with immuno-oncologic agents once safety profiles are established.

The Akt/mTOR pathway is active in HNSCC; mTOR inhibitors display biologic activity in preclinical and clinical HNSCC models, and currently available oral mTOR inhibitors are considered well-tolerated for prolonged periods up to four years.[43, 44] Moreover, no increased incidence of immunosuppression has been observed in multiple trials of single-agent rapamycin or rapamycin analogues in cancer patients.[45, 46] Clinical trials have shown that mTOR inhibitors have clinical activity in HNSCC.[11, 12, 47] We have evidence that mTOR inhibitors hold promise for minimal residual disease in preclinical models.[48-50] mTOR inhibitors have been safely used in transplant patients for years without significant side effects. This study may represent evidence that p16-negative patients and those patients with *TP53mut* could potentially benefit from mTOR inhibitors as adjuvant therapy. The 50% of advanced stage patients that recur will do so in the first two years of current standard treatment. Molecular analysis of *TP53* in surgical margins has shown it to be a likely predictor of recurrence.[51] Many studies have shown that mutant *TP53* promotes sustained activation of the PI3K/Akt/mTOR pathway in cancer and results in autophagy inhibition.[6, 52, 53] A possible mechanism of action for everolimus may be its ability to inhibit this activation, resulting in a cell death response. A Phase II trial randomizing p16-negative and/or *TP53mut* patients to everolimus and placebo for at least two years will determine if this drug can change survival in a group of patients that have the worst outcomes. Finally, given the growing cost of health care, rapamycin, which has been shown to have the same pharmacodynamic activity as sirolimus and temsirolimus[54-56], is an inexpensive drug that is safe and could improve PFS in patients who recur within the first two years.

LIMITATIONS:

A key limitation of this trial is that it was underpowered due to study closure prior to complete accrual. Feasibility challenges included engaging patients in maintenance therapy after definitive treatment, randomization to placebo, and factors such as PI change of institution, which impeded routine study support and function. Although investigators were unlikely to have unconscious bias due to the drug administration structure, the risk exists for inadvertent unblinding due to recognizable side effects during follow up. There was a chance baseline difference in T staging among groups where the most common stage in the everolimus group was T4A or 4B (57%) compared to mostly T2 patients who received placebo (42%); yet, despite this imbalance we were still able to observe a survival benefit with everolimus in p16-negative patients. The data strongly support conducting a large trial randomizing high-risk p16-negative patients with *TP53mut* to everolimus or placebo as adjuvant therapy. Additionally, as patients were permitted to enroll up to 16 weeks after definitive treatment, the possibility of selection bias for high-risk relapse exists. Moreover, extending adjuvant therapy with everolimus administration beyond one year has the potential to further improve PFS for HNSCC patients with the historically most unfavorable prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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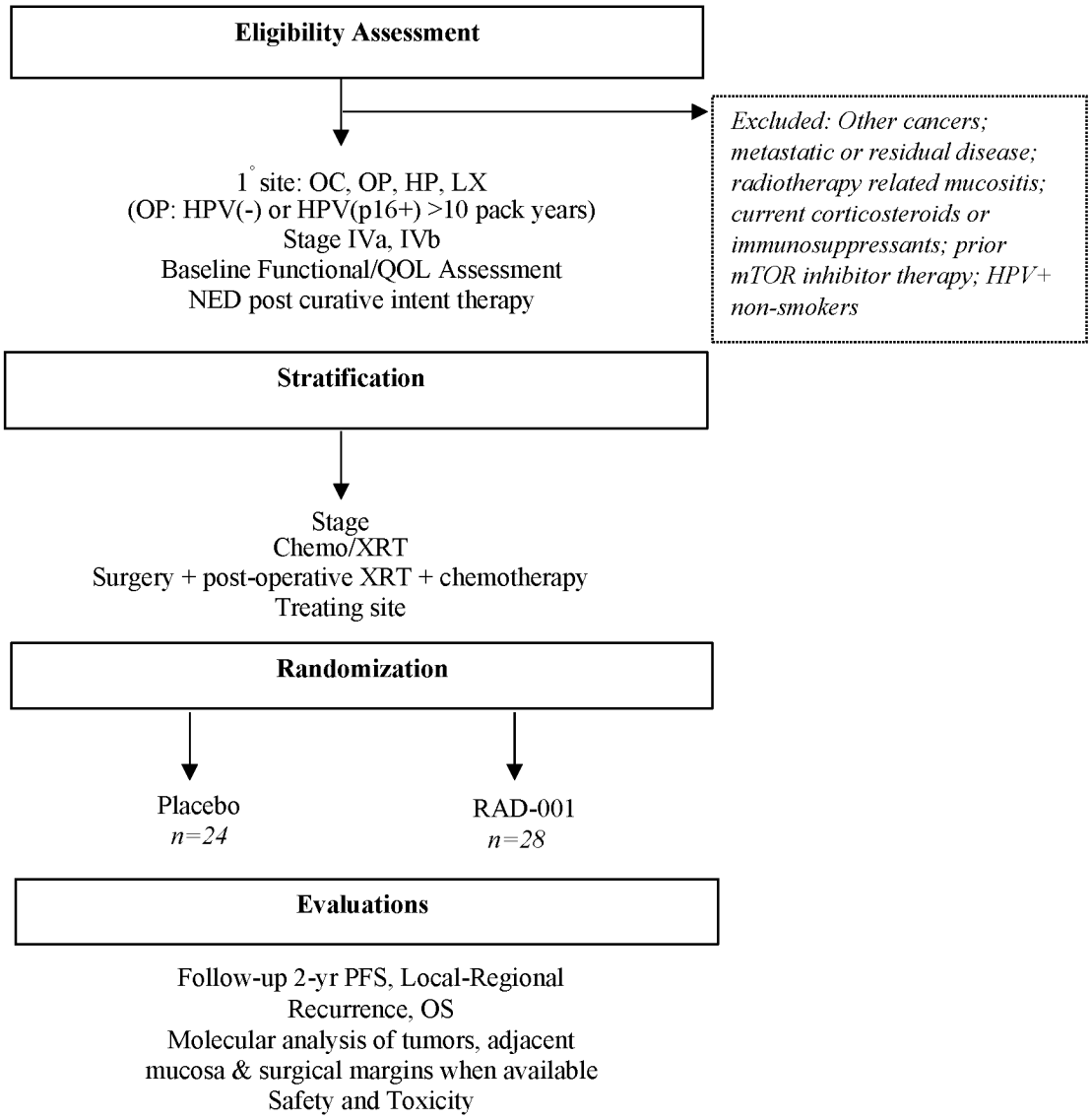
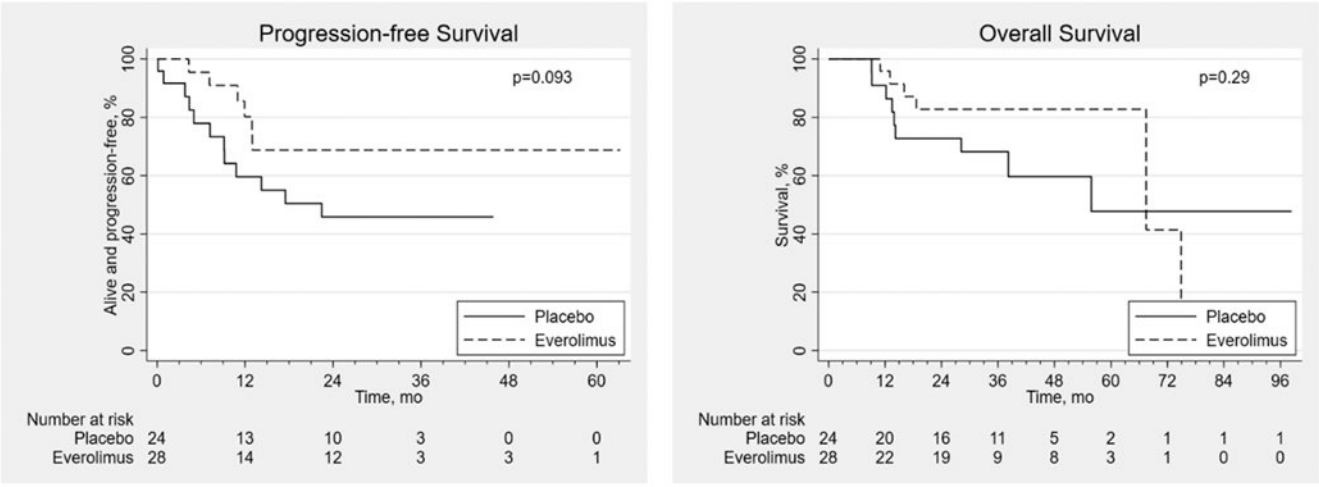


Figure 1. Summary of study design.

Abbreviations: OC=Oral Cavity, OP=Oropharynx, HP=Hypopharynx, LX=Larynx, HPV=Human Papillomavirus, QOL=Quality of Life, NED=No evidence of disease, XRT: Radiotherapy



2-year PFS rates:

Everolimus: 68.8% (95% CI: 47.7% to 89.8%)
Placebo: 45.8% (95% CI: 25.0% to 66.7%)
Diff: 22.9% (95% CI: -6.7% to 52.1%)
(p=0.13)

Figure 2. Progression-free and overall survival in patients receiving everolimus (E) (n=28) versus placebo (P) (n=24).

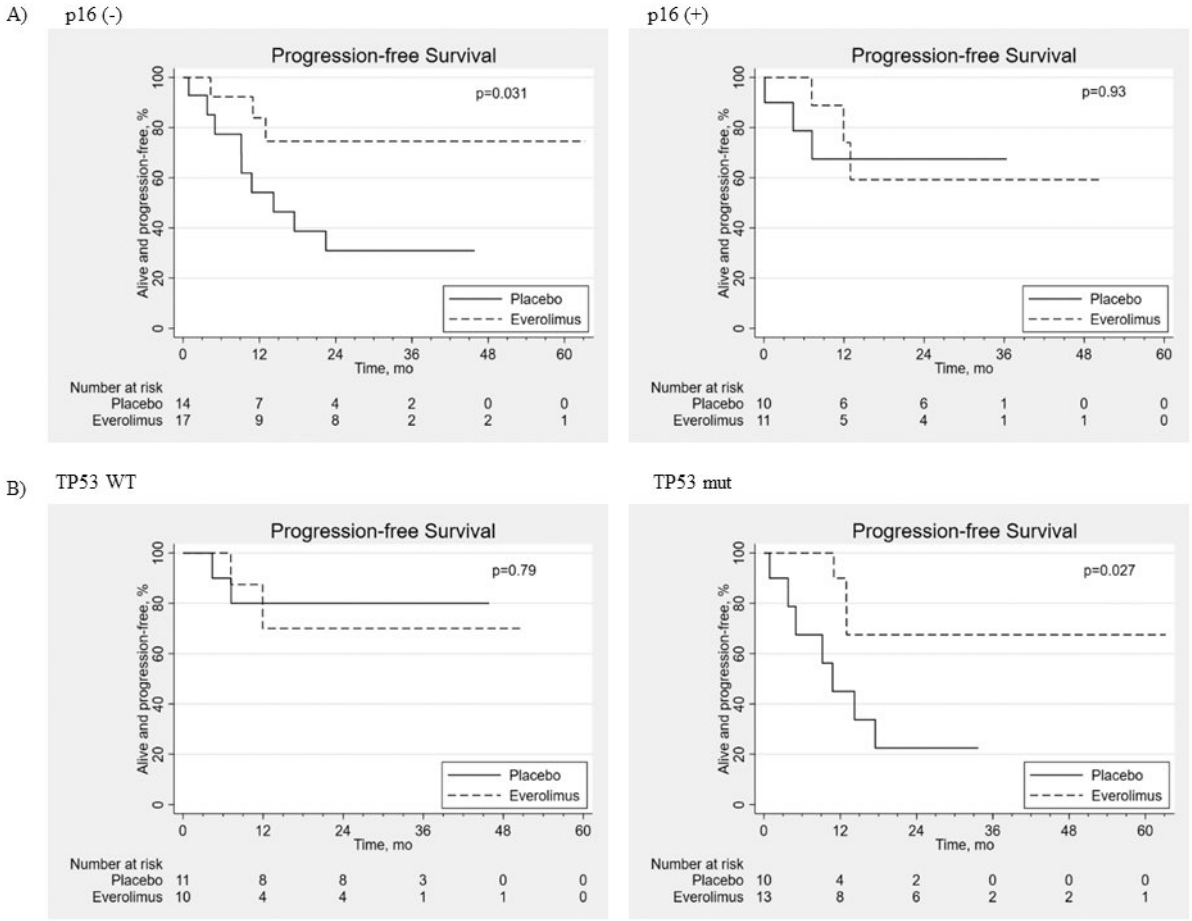


Figure 3. Progression-free survival in patients by p16 and TP53 status.

(A) Progression-free survival in p16 (−) (n=31) and p16 (+) (n=21) patients receiving everolimus (E) versus placebo (P). (B) Progression-free survival in *TP53* wild-type (WT) (n=21) and *TP53* mutant (mut) (n=23) patients receiving everolimus (E) versus placebo (P).

Table 1.

Patient sociodemographic and clinical characteristics, post randomization.

Variable	Placebo (n = 24)	Everolimus (n = 28)
Age (years), mean (SD)	57.3 (8.4)	58.8 (7.3)
Gender		
Male	20 (83%)	23 (82%)
Female	4 (17%)	5 (18%)
Race		
African-American	3 (12%)	3 (11%)
Caucasian	21 (88%)	25 (89%)
Tumor primary site		
Oral Cavity	5 (21%)	6 (21%)
Oropharynx	14 (58%)	14 (50%)
Larynx	1 (4%)	7 (25%)
Hypopharynx	4 (17%)	1 (4%)
Tumor stage		
T1	0 (0%)	5 (18%)
T2	10 (42%)	3 (11%)
T3	6 (25%)	4 (14%)
T4A or 4B	8 (33%)	16 (57%)
Nodal stage		
N0	2 (8%)	3 (11%)
N1	1 (4%)	1 (4%)
N2	4 (17%)	4 (14%)
N2B	9 (38%)	6 (21%)
N2C	7 (29%)	11 (39%)
N3	1 (4%)	3 (11%)
Karnofsky Performance Score		
100	5 (21%)	5 (18%)
90	9 (38%)	13 (46%)
80	9 (38%)	9 (32%)
Unknown	1 (4%)	1 (4%)
HPV (p16 or ISH) result		
Negative	14 (58%)	16 (57%)
Positive	10 (42%)	12 (43%)
***TP53* mutation status**		
Wild type	13 (54%)	15 (54%)
Mutated	10 (42%)	13 (46%)
Days from end of definitive treatment to study drug initiation, mean (SD)	93 (30)	115 (97)
Pack-years smoking, mean (SD)	31.9 (24.8)[1]	48.5 (32.4)[2]

Variable	Placebo (n = 24)	Everolimus (n = 28)
Treatments administered		
Surgery	10 (42%)	15 (54%)
RT	23 (96%)	23 (82%)
Induction chemotherapy	4 (17%)	5 (18%)

[1] 12 missing observations

[2] 16 missing observations

Table 2.

Adverse events at least possibly related to study drug occurring in at least 5% of patients, by NIH-NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Adverse Event by Grade Everolimus				
Adverse Event	**Worst Grade (No, %)**			
	1	**2**	**3**	**4**
Anorexia	4 (14%)	2 (7%)	0	0
Cholesterol high	4 (14%)	1 (4%)	0	0
Creatinine increased	1 (4%)	1 (4%)	0	0
Diarrhea	2 (7%)	0	1 (4%)	0
Dysgeusia	1 (4%)	1 (4%)	0	0
Fatigue	2 (7%)	3 (11%)	1 (4%)	0
Headache	1 (4%)	0	1 (4%)	0
Hypertriglyceridemia	0	2 (7%)	2 (7%)	0
Lymphocyte count decreased	2 (7%)	4 (14%)	1 (4%)	0
Nausea	2 (7%)	1 (4%)	0	0
Neutrophil count decreased	0	1 (4%)	1 (4%)	0
Papulopustular rash	3 (11%)	0	0	0
Platelet count decreased	4 (14%)	2 (7%)	0	0
Rash acneiform	1 (4%)	0	1 (4%)	0
Rash maculo-papular	1 (4%)	1 (4%)	0	0
Skin and subcutaneous tissue disorders	1 (4%)	1 (4%)	0	0
Skin infection	0	1 (4%)	*1 (4%)	0
Stomatitis/pharyngitis	3 (11%)	7 (25%)	2 (7%)	0
White blood cell decreased	4 (14%)	3 (11%)	1 (4%)	0

*Serious adverse event requiring hospitalization