Investigational New Drugs (2019) 37:702–710
https://doi.org/10.1007/s10637-018-0696-4

PHASE I STUDIES

A phase 1 trial of Vorinostat in combination with concurrent chemoradiation therapy in the treatment of advanced staged head and neck squamous cell carcinoma

Theodoros N. Teknos[1,2] · J. Grecula[3] · A. Agrawal[1] · M. O. Old[1] · E. Ozer[1] · R. Carrau[1] · S. Kang[1] · J. Rocco[1] · D. Blakaj[3] · V. Diavolitsis[3] · B. Kumar[1] · P. Kumar[1] · Q. Pan[1] · M. Palettas[4] · L. Wei[4] · R. Baiocchi[5] · P. Savvides[5]

Received: 4 June 2018 / Accepted: 12 November 2018 / Published online: 19 December 2018

Summary

Purpose Vorinostat is a potent HDAC inhibitor that sensitizes head and neck squamous cell carcinoma (HNSCC) to cytotoxic therapy while sparing normal epithelium. The primary objective of this Phase I study was to determine the maximally tolerated dose (MTD) and safety of Vorinostat in combination with standard chemoradiation therapy treatment in HNSCC. *Patients and Methods* Eligible patients had pathologically confirmed Stage III, IVa, IVb HNSCC, that was unresectable or borderline resectable involving the larynx, hypopharynx, nasopharynx, and oropharynx. Vorinostat was administered at the assigned dosage level (100-400 mg, three times weekly) in a standard 3 + 3 dose escalation design. Vorinostat therapy began 1 week prior to initiation of standard, concurrent chemoradiation therapy and continued during the entire course of therapy. *Results* Twenty six patients met eligibility criteria and completed the entire protocol. The primary tumor sites included tonsil (12), base of tongue (9), posterior pharyngeal wall (1), larynx (4) and hypopharynx (3). Of the 26 patients, 17 were HPV-positive and 9 were HPV-negative. The MTD of Vorinostat was 300 mg administered every other day. Anemia ($n = 23/26$) and leukopenia ($n = 20/26$) were the most commonly identified toxicities. The most common Grade3/4 events included leukopenia ($n = 11$) and lymphopenia ($n = 17$). No patient had Grade IV mucositis, dermatitis or xerostomia. The median follow time was 33.8 months (range 1.6–82.9 months). Twenty four of 26 (96.2%) patients had a complete response to therapy. *Conclusion* Vorinostat in combination with concurrent chemoradiation therapy is a safe and highly effective treatment regimen in HNSCC. There was a high rate of complete response to therapy with toxicity rates comparable, if not favorable to existing therapies. Further investigation in Phase II and III trials is strongly recommended.

Keywords Head and neck cancer · Oropharyngeal cancer · Chemoradiation therapy · Organ preservation · Phase I trial in advance stage head and neck cancer · Histone deacetylase inhibitors in head and neck cancer · HPV-related head and neck cancer

Background

Head and neck squamous cell carcinoma (HNSCC) is the 8th leading cancer worldwide with almost 650,000 new cases diagnosed every year and 350,000 cancer-related deaths annually [1–3]. The worldwide incidence of HNSCC is also increasing due to the emerging epidemic of human papillomavirus (HPV) related head and neck cancer. Two distinct clin-

✉ Theodoros N. Teknos
Theodoros.Teknos@UHHospitals.org

[1] Otolaryngology, The Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC – James), Columbus, OH, USA

[2] Seidman Cancer Center, University Hospitals Cleveland Medical Center, Cleveland, OH, USA

[3] Radiation Oncology, The Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC – James), Columbus, OH, USA

[4] Center for Biostatistics, The Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC – James), Columbus, OH, USA

[5] Hematology-Medical Oncology, The Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC – James), Columbus, OH, USA

ical and molecular entities of HNSCC have been identified based on the presence or absence of HPV in the tumor. "Classic" or HPV-unrelated malignancies typically occur in individual who abuse tobacco, alcohol and have poor oral hygiene [4]. Despite advances in surgery, radiation and chemotherapy, the overall survival rates for HPV-unrelated cancers have not improved significantly over the last several decades and disease specific survival remains at or below 50% [5, 6]. Conversely, HPV-related HNSCC frequently occurs in nonsmokers and has far superior survival rates under most circumstances [7]. Historically, surgery and external beam radiation therapy had been the mainstay of treatment for advanced HNSCC, but even with the latest reconstructive techniques, surgical morbidity remains high [8–10]. The desire to improve functional and oncologic outcomes led to several landmark studies illustrating the feasibility and efficacy of concurrent chemoradiation therapy regimen in lieu of surgery for the treatment of advance stage HNSCC [11–15]. Despite the near equivalent oncologic outcomes of these "organ sparing" approaches, the toxicities resulting from these regimen are significant. The incidence of osteoradionecrosis, peripheral neuropathy, significant hearing loss, swallowing dysfunction and gastrostomy tube dependence vary widely in the literature but remain a significant problem in this patient population. As a result, there has been intense interest in identifying novel biologic agents that enhance sensitivity to cytotoxic therapy while minimizing side effects to normal mucosa [16, 17].

Recently, histone deacetylase inhibitors (HDACi) have been identified as a potential targeted therapy agent due to the selective expression of histone deactylase (HDAC) in neoplasms. HDACs are known to alter chromatin configuration and regulate numerous genes involved in cancer initiation and progression [18–24]. Specifically, HDACs remove acetyl groups from histones creating non-permissive chromatin and suppress gene transcription [25]. Importantly, most of the genes repressed by HDACs are tumor suppressors, cell cycle regulators and inducers of apoptosis [25–30]. Furthermore, HDACs deacetylate and inactivate a number of proteins and transcription factors, post-translation [31–34]. In humans, 18 HDACs have been identified and grouped into four classes [35, 36]. Several inhibitors of HDACs have been developed in the last decade and many are in clinical trials for both solid and hematologic malignancies [37–41]. In both in-vitro and in-vivo studies, HDACi comprise a promising category of targeted therapeutic agents with potential for cell cycle disruption, inhibition of angiogenesis, inhibition of cell proliferation and induction of apoptosis in cancer cells but not in normal tissues [41–45]. HDACi have also been shown to reverse resistance to cisplatin therapy, sensitize tumors to radiation therapy, specifically target the cancer stem cell population through the down-regulation of nanog while protecting normal structures (including the skin) from radiation injury [46–49]. Specific to the HPV related malignancies, HDACi have been shown to improve antigen presentation and elicit an HPV specific T cell responses [50, 51]. Vorinostat [suberoylanalide hydroxamic acid (SAHA), Zolinza®,L001079038, Merck and Co.] is a pan-HDAC inhibitor which has been shown to be well tolerated in clinical trials. Due to the frequent resistance to therapy noted in HPV-unrelated HNSCC, the immunologic sequellae of HPV related tumors and the significant toxicities reported by all HNSCC patients, a novel Phase I trial was designed to identify the maximally tolerated dose (MTD) of Vorinostat in patients with unresectable or borderline resectable laryngeal, hypopharyngeal, nasopharyngeal and oropharyngeal carcinoma being treated with standard fractionation external beam radiotherapy combined with cisplatin chemotherapy.

Patients and methods

Patient eligibility

All eligible patients had: 1) pathologically confirmed HNSCC, 2) Stage III, Stage IVa or Stage IVb disease, 3) unresectable or borderline resectable disease involving the larynx, hypopharynx, nasopharynx and oropharynx. Patients were deemed unresectable if they have clinically confirmed carotid artery encasement, skull base involvement, trismus or deep neck musculature invasion at the time of diagnosis. Borderline resectability was defined as those patients in which surgery was unlikely to result in excision of all macroscopic disease or would result in a total glossectomy, total laryngopharyngectomy, skull base resection or carotid resection. All patients were required to be >18 years of age, be able to provide written consent, have an ECOG performance status of 0–2 and have adequate hematologic, renal and hepatic function. Ineligibility criteria included: 1) major surgery or trauma within 28 days of enrollment 2) prior radiation to any of the planned radiation field, 3) history of hypersensitivity to HDACi or any other drug used in this study 4) active peptic ulcer disease 5) pregnant or lactating women 6) uncontrolled co-morbid conditions that would limit compliance with the treatment regimen and 7) patients with active viral hepatitis or known HIV infection.

Dosage and drug administration

Vorinostat was supplied in 100 mg gelatin capsules and patients were instructed to swallow the capsules whole with food. For those who were unable to swallow the capsules due to dysphagia or mucositis, gastrostomy tube administration of a Vorinostat suspension was allowed according to a specific recipe that was formulated and tested for efficacy and dose equivalency. Subjects were instructed to keep a pill diary

and return the empty bottle to confirm compliance with therapy. Vorinostat was administered at the assigned dosage level (100-400 mg) three times weekly. Vorinostat therapy began 1 week prior to the initiation of radiation and cisplatin therapy and continued during the entire course of chemoradiation. Cisplatin (100 mg/m^2) was administered during weeks 1, 4 and 7 of radiation therapy. Intensity modulated radiation therapy (IMRT) planning and delivery was performed in all cases. Radiation therapy was given over a 7 week period, 2Gy per fraction per day, 5 days a week to a maximum of 70.2Gy. The lymph node groups at risk were determined and their volumes were outlined on the treatment planning CT according to image-based nodal classifications (RTOG online nodal atlas can be used as a reference; www.rtog.org). The suggested dose restraints for critical normal structures included: 1) brainstem: </= 54 Gy, 2) spinal cord: </= 45Gy, 3) mandible: </= 70 Gy, 4) mean dose to contralateral parotid gland </=26 Gy or at least 50% of parotid </=30 Gy 5) Brachial plexus received no more than 5% >60 Gy but must be </=66 Gy, 6) Cochlea: no more than 10% received >40 Gy, 7) >1% of unspecified tissue outside the targets should <110% of the prescribed dose to PTV, 8) posterior neck received no more than 20% >40 Gy.

Dose escalation design

This trial utilized the standard 3 + 3 dose escalation design with Vorinostat being escalated while radiation and cisplatin doses were held constant. Dose escalation was not considered until at least three evaluable patients had been been observed for 28 days following the completion of chemoradiation therapy. The doses of Vorinostat studied included 100 mg MTW, as well as 100 mg, 200 mg, 300 mg and 400 mg MWF. Intrapatient dose escalation was not allowed. The maximally tolerated dose (MTD) was defined as the dose in which fewer than 2 of 6 patients experienced dose limiting toxicities (DLT). DLT's were defined as any of the following events: 1) febrile neutropenia with or without infection (grade 3 or 4), 2) grade 4 neutropenia lasting for >7 days, 3) grade 4 thrombocytopenia, 4) any grade 5 hematologic toxicity 5) grade 3–4 non-hematologic toxicity attributed to chemotherapy and/or vorinostat excluding grade 3 mucositis and ototoxicity. Nausea and vomiting were considered dose-limiting only if they were not controlled with adequate antiemetic therapy. 6) any toxicity occurring in cycle 1 that resulted in a dose reduction in vorinostat. Consecutive doses of Vorinostat may be missed after Cycle 1 but five or more doses missed will be considered a DLT 7) mucositis will not be considered dose limiting unless it fails to resolve to grade 2 with 10 day or less treatment break 8) diarrhea that is Grade 3 and persists after the initiation of antidiarrheal therapy. 9) Grade 3 or 4 electrolyte abnormality not correctable by replacement.

Pre-treatment and follow up studies

Within 28 days of beginning therapy, patients had a complete medical history, physical examination, vital signs, assessment of ECOG performance status, dental evaluation, nutritional evaluation, tumor volume assessment via imaging, CBC w/diff, comprehensive serum chemistry panel, PTT, EKG and pregnancy test for female patients. During the 1 week run-in period of Vorinostat, patients were examined for toxicities and CBC w/diff, comprehensive serum chemistry panel and PTT were drawn. During the 7 weeks in which the patients were receiving Vorinostat in combination with cisplatin/radiation therapy, they were seen weekly by a physician and toxicities were assessed using standard NCI-CTCAEv4.03 criteria. A complete physical exam, vitals, performance status assessment, CBC w/diff, comprehensive serum chemistry panel and PTT were drawn. Tumor response to therapy was assessed by the surgical team during weeks four and seven of concurrent chemoradiation

Table 1 Patient demographics

	Total (n = 26)
Age [Median (SD)]	56.5 (8.3)
Race [n (%)]	
Black	2 (7.7)
White	24 (92.3)
Gender [n (%)]	
Male	23 (88.5)
Cigarette use [n (%)]	
Yes	18 (69.2)
No	8 (30.8)
T staging [n (%)]	
2	9 (34.6)
3	12 (46.2)
4	5 (19.2)
N staging [n (%)]	
0	2 (7.7)
1	2 (7.7)
2	19 (73.1)
3	3 (11.5)
M staging [n (%)]	
0	26 (100.0)
Overall staging [n (%)]	
III	4 (15.4)
IV	22 (84.6)
HPV positive [n (%)]	
Yes	17(65.4)
No	9(34.6)
P16 positive [n (%)]	
Yes	17 (65.4)
No	8 (30.8)
Unknown	1 (3.9)

therapy. Four weeks following the completion of therapy, toxicities were once again assessed, blood samples were drawn and tumor response to therapy was reassessed.

Statistical analysis

Patient characteristics and responses were summarized using descriptive statistics. Toxicities were also summarized by grade per the NCI CTCAE v4.03 criteria using frequency and percentage. Overall survival (OS) was calculated from the start of treatment to death from any cause. Patients who were still alive were censored at the date of last follow up. Disease free survival (DFS) was calculated from the start of treatment to disease recurrence. Patients who were disease free were censored at the date of last follow up. Survivals were estimated using the method of Kaplan-Meier. Statistical analysis was performed using SAS version 9.4 for Windows (SAS Institute, Cary, NC).

Results

Study population

Twenty nine previously untreated Stage III, IVa and IVb patients consented to the clinical trial and 26 completed the entire protocol. Two patients withdrew prior to beginning therapy and one patient withdrew after receiving a single dose of Vorinostat. The primary tumor sites included tonsil ($n = 12$), base of tongue ($n = 9$), posterior pharyngeal wall (n = 1), supraglottic larynx ($n = 2$), glottic larynx (n = 2) and hypopharynx ($n = 3$). Table 1 summarizes the demographic and clinical data of the study population. Of the HPV positive patients ($n = 17$), 13 were smokers with greater than a 10 pack year history.

Safety and maximally tolerated dose

Table 2 summarizes the most common adverse events that were deemed to be at least "possibly related" to the study therapy. Anemia was the most common adverse event ($n = 23$), followed by leukopenia ($n = 20$). The most common ≥Grade 3 adverse events included leukopenia ($n = 11$) and lymphopenia (n = 17). Overall, the treatment regimen was well tolerated with a mean weight loss of 11.69% (range 0.79–20.81%), 3/26 patients requiring radiation treatment breaks (all less than 5 days in duration), 0/26 patients developing osteoradionecrosis (ORN), and only 1/26 patients developing Grade 3 xerostomia. Figure 1 illustrates the rates of Grade 3/4 mucositis, radiation dermatitis and weight loss. Dysphagia and pharyngeal stricture occurred in 4/26 patients. All of these patients returned to normal swallowing function after a single dilation procedure ($n = 3$) or two dilation procedures ($n = 1$). All patients who have completed this protocol are able to take a full oral diet and 0/26 have required long term G-tube alimentation.

Four dosing levels of Vorinostat were given in combination with standard cisplatin and IMRT radiation therapy: 100 mg MTW, 100 mg MWF, 200 mg MWF, 300 mg MWF and 400 mg MWF. Two of five patients in the 100 mg MTW developed DLT's. The dosing schedule was then adjusted to every other day (MWF) with much better tolerability. Figure 2 illustrates the number of DLT's per dosing cohort and identifies 300 mg Vorinostat as the maximally tolerated dose.

Table 2 Adverse event toxicity frequencies

AE toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Total
Hemoglobin	13	9	1	0	23
Leukocytes (total WBC)	3	6	9	2	20
Mucositis/stomatitis (clinical exam) - Oral cavity	5	7	8	0	20
Dry mouth/salivary gland (xerostomia)	17	1	1	0	19
Lymphopenia	0	3	7	10	20
Nausea	10	5	4	0	19
Sodium, serum-low (hyponatremia)	11	0	9	0	20
Fatigue (asthenia, lethargy, malaise)	11	7	0	0	18
Weight loss	3	11	4	0	18
Dysphagia (difficulty swallowing)	8	8	1	0	17
Platelets	13	3	0	0	16
Taste alteration (dysgeusia)	8	9	0	0	17
Glucose, serum-high (hyperglycemia)	12	2	1	1	16
Constipation	8	6	0	0	14
Vomiting	10	3	1	0	14
Albumin, serum-low (hypoalbuminemia)	6	6	0	0	12

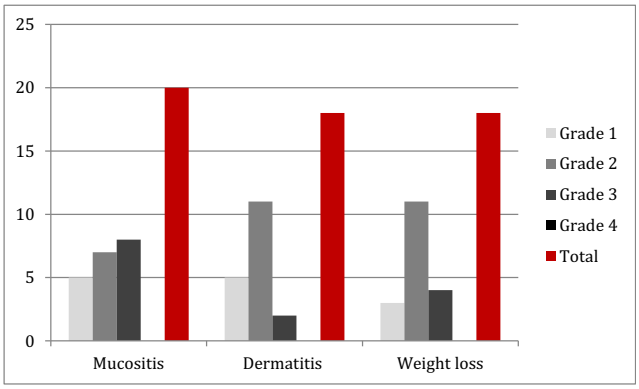


Fig. 1 The incidence and grade of mucositis, dermatitis and weight loss for the patients in this trial

Response to therapy

The median follow up time for the patients enrolled on this trial was 33.8 months (range 1.6–82.9 months). Twenty five of twenty six patients (96.2%) had a complete response to therapy. To date only three patients (11.5%) had a recurrence of disease. A total of four patients in this cohort died; two of recurrent disease and two of other causes but free from HNSCC. One patient is alive with metastatic disease to the lungs at 27.3 months follow up. Figure 3 illustrates the estimated 5 year overall survival rate at 68.4% and the median overall survival was not reached (95% CI: 52.6 months – not reached). The estimated 5 year disease free survival rate is 76.6% and the median disease free survival was not reached (95% CI: 52.6 months – not reached).

Discussion

This is the first clinical trial evaluating the use of Vorinostat in combination with concurrent chemoradiation therapy for the treatment of advanced stage, unresectable HNSCC. In this Phase I trial, 300 mg every other day dosing of Vorinostat was identified as the MTD and a favorable toxicity profile was established. Although clinical response to therapy was not the primary endpoint of this study, responses to this treatment regimen have been very encouraging to date. The rate of CR (96.2%), estimated 5 yr. overall survival (68.4%) and 5 yr. disease-free survival (76.6%) are remarkable in light of the fact that a variety of head and neck tumor biology was represented in this patient cohort (HPV-related HNSCC = 17/26, Tobacco abuse = 18/26, Larynx/Hypopharynx primary tumors = 7/26). The estimated 5 year overall survival in this study (68.4%) which included both HPV positive and negative patients compare favorably to similar patient cohorts treated with cisplatin and radiation therapy alone (overall survival in HPV positive and negative patients, 70.9% and 46.2% respectively) [7].

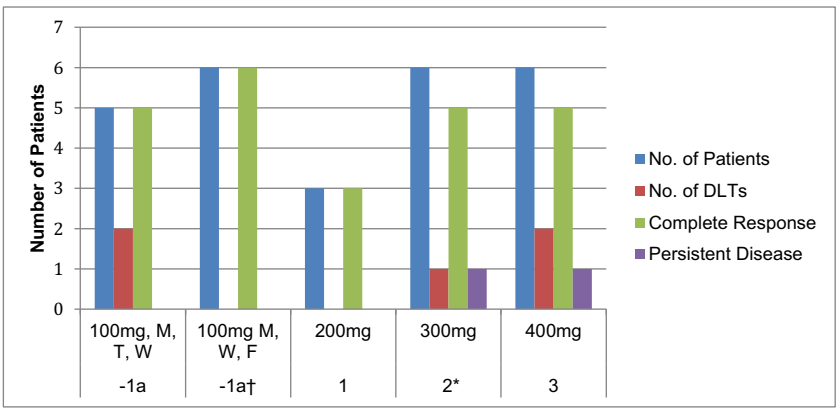
Other important findings gleaned from this investigation included the development and validation of a Vorinostat suspension that allowed for equivalent dosing through both G-tube and oral routes of administration (Figs. 4, 5 and 6).

Growing evidence indicates that more aggressive chemoradiation therapy improves local control and survival in patients with HNSCC [52]. However, better outcomes have come at the expense of increased morbidity and mortality. The rates of mucositis, radiation dermatitis, weight loss, peripheral neuropathy and hearing loss in the acute setting, as well as pharyngeal/laryngeal dysfunction, feeding tube

Fig. 2 The number of DLT's per dosing cohort, treatment response and the identification of 300 mg MWF as the MTD

Dose Level	Vorinastat (mg)	No. of Patients	No. of DLTs	Complete Response	Persistent Disease
-1a	100mg, M, T, W	5	2	5	0
-1a†	100mg M, W, F	6	0	6	0
1	200mg	3	0	3	0
2*	300mg	6	1	5	1
3	400mg	6	2	5	1

*Maximally Tolerated Dose



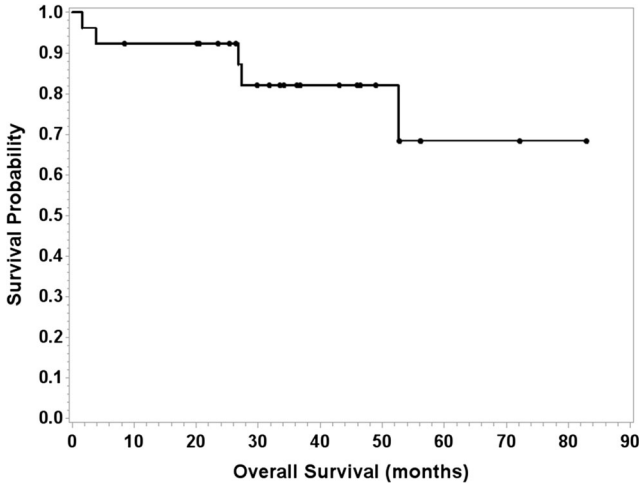


Fig. 3 Five year overall survival

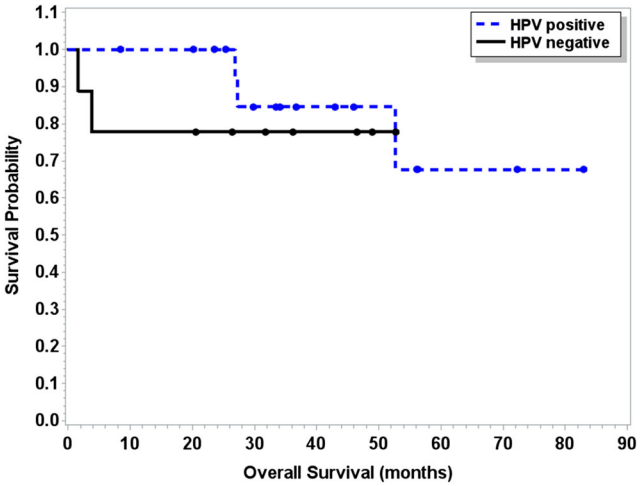


Fig. 5 Five year overall survival by HPV group

dependence and osteoradionecrosis in the chronic setting are unacceptably high [53–57]. The concept of adding another therapeutic agent to this already toxic regimen is a difficult one to justify. In fact, recent investigations, including RTOG-0552: A Randomized Phase III Trial of Concurrent Accelerated Radiation Plus Cisplatin with or without Cetuximab, demonstrated no improvement in 3 yr. OS or PFS but the Cetuximab arm resulted in more frequent interruptions in radiation (26.9% vs 15.1%) and increased the Grade 3/4 toxicity rates for mucositis (43.2% vs. 33.3%), rash, fatigue and hypokalemia [58]. In addition, single agent SAHA has not been found to be effective against head and neck cancer as a single agent in the recurrent/metastatic disease setting [59–62]. However, because HDAC inhibitors result in a relaxed chromatin configuration, they create a more permissive environment for DNA double strand breaks to occur when malignant cells are exposed to cytotoxic therapy [63]. As a result, studies have utilized SAHA in combination with chemotherapy for HNSCC and shown that these are well tolerated and result in tumor response [64–66]. SAHA has also been shown to be a potent radiosensitizing agent in both preclinical and clinical investigations [67–70]. In addition to these observations, HDACi have been found to: 1) selectively target radiation resistant cancer stem cells, 2) decrease tumor inflammation, 3) down-regulate Bcl-2, NFkB, EGFR and AKT expression, 4) improve antigen presentation and immune recognition of tumor, 5) increase the HPV-E7 specific T cell response, 6) abolish osteoclastogenesis and 7) decrease cancer cachexia and sarcopenia [46, 51, 71–75]. These data provide a strong rationale for this trial.

The most intriguing results of this trial are the tumor response rates coupled with a toxicity profile that is comparable to, if not superior, to chemoradiation therapy alone. All patients on this trial were able to complete radiation without interruption and their mean weight loss was 11.69%. While 74.1% (20/27 patients) experienced mucositis, only 29.6% (8/27) developed Grade 3 mucositis and no patient reached a Grade 4 level. These results compare favorably to systematic reviews of the

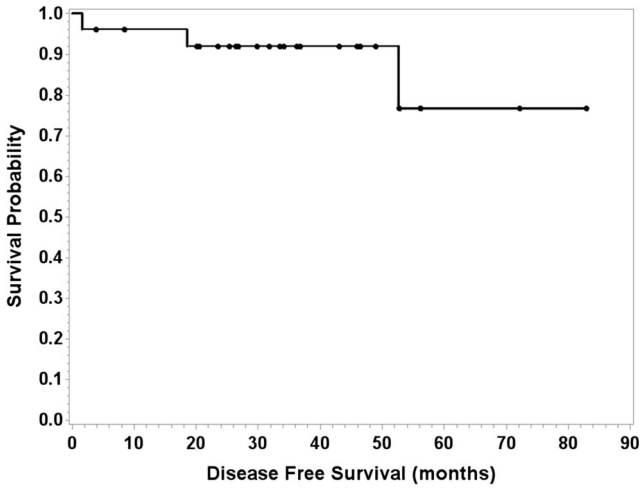


Fig. 4 Five year disease free survival

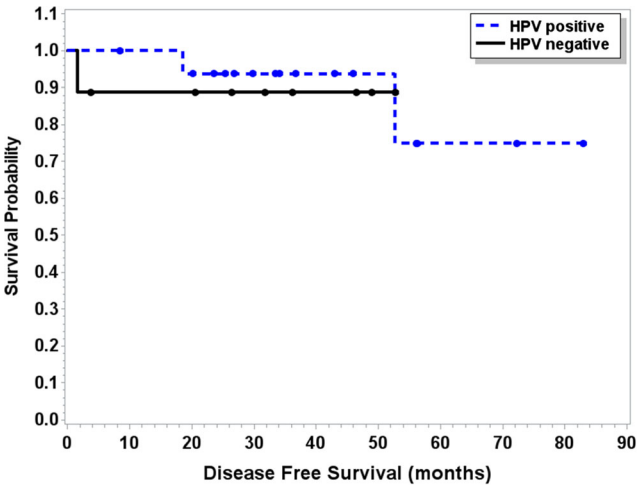


Fig. 6 Five year disease free survival by HPV group

literature reporting an average of 15% of patients requiring treatment breaks during radiation, average wt. loss of >10%, Grade 3/4 mucositis rates of 43%, 69% rates of oral pain and 53% opioid use [54]. The low rate of radiation related dermatitis (16/27 Grade 1/2, 1/27 Grade 3) corroborates preclinical investigations in which TNFa mediated dermatitis could be minimized with this regimen. Finally, post-treatment laryngeal and pharyngeal dysfunction is frequently encountered in this patient population with studies reporting as high as a 30% rate of Gtube use, 1 year following therapy and an 85% rate of swallowing dysfunction on video swallowing studies [76]. Despite a 14.81% rate of pharyngeal stricture (4/27 patients) the patients in this study responded well to esophageal dilation and all were able to tolerate a regular diet at last follow up.

With regard to tumor response to therapy, the results are encouraging but must be assessed with caution. The majority of patients were HPV positive (17/26, 65.4%) and it is well documented that these patients have superior survival outcomes. However, the fact that this trial selected advanced, unresectable tumors, with large volume disease (22/26, 84.6% N2, N3), significant smoking history (18/26, 69.2% smokers) and non-oropharyngeal subsites (7/27, 25.92%) indicates that further investigation, in the form of a Phase II clinical trial, is warranted.

In summary, this investigation is the first of its kind, combining SAHA with standard chemoradiation therapy for advanced stage head and neck squamous cell carcinoma. The maximally tolerated dose of Vorinostat was 300 mg given every other day beginning 1 week prior to and continuing throughout the entire 7 weeks of chemoradiation therapy. The regimen was well tolerated and effective in this patient population. A novel gastrostomy tube formulation of Vorinostat was created and validated for equivalency during the course of this Phase I trial as well. Due to the encouraging results obtained with regard to toxicity and tumor response, additional investigations regarding mechanism of action and clinical utility are underway.

Acknowledgements This study was approved and funded by the National Comprehensive Cancer Network (NCCN) Oncology Research Program from general research support provided by Merck & Co., Inc.

Funding This study was funded by the National Comprehensive Cancer Network (NCCN) Oncology Research Program from general research support provided by Merck & Co., Inc.

Compliance and ethical standards

Conflict of interest All authors declare that there are no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Leemans CR, Braakhuis BJM, Brakenhoff RH (2011) The molecular biology of head and neck cancer. Nat Rev Cancer 11(1):9–22
2. Mehanna H, Paleri V, West CML, Nutting C (2010) Head and neck cancer-part 1: epidemiology, presentation, and prevention. BMJ 341:c4684
3. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A (2016) Cancer treatment and survivorship statistics. Cancer J Clin 66:271–289
4. Chaturvedi AK, Graubard BI, Broutian T, Pickard RKL, Tong ZY, Xiao W, Kahle L, Gillison ML (2015) NHANES 2009-2012 findings: association of sexual behaviors with higher prevalence of oral oncogenic human papillomavirus infections in U.S. men. Cancer Res 75:2468–2477
5. Kalavrezos N, Bhandari R (2010) Current trends and future perspectives in the surgical management of oral cancer. Oral Oncol 46:429–432
6. Edwards BK, Brown ML, Wingo PA et al (2005) Annual report to the nation on the status of cancer, 1975–2002, featuring population based trends in cancer treatment. J Natl Cancer Inst. Oct 5 97(19): 1407–1427
7. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML (2010) Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 363:24–35
8. Carvalho AL, Hishimoto IN, Califano JA et al (2005) Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer 114:806–881
9. Preuss SF, Quante G, Semrau R, Mueller RP, Klussmann JP, Guntinas-Lichius O (2007) An analysis of surgical complications, morbidity, and cost calculation in patients undergoing multimodal treatment for operable oropharyngeal carcinoma. Laryngoscope 117(1):101–105
10. Sundaram K, Schwartz J, Har-El G et al (2005) Carcinoma of the oropharynx: factors affecting outcome. Laryngoscope 115(9): 1536–1542
11. Wolf GT, Fisher SG, Hong WK et al (1991) 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 324:1685–1690
12. Urba S, Wolf G, Eisbruch A, Worden F, Lee J, Bradford C, Teknos T, Chepeha D, Prince M, Hogikyan N, Taylor J (2006) Single-cycle induction chemotherapy selects patients with advanced laryngeal cancer for combined chemoradiation: a new treatment paradigm. J Clin Oncol 24:593–598
13. Worden FP, Kumar B, Lee JS et al (2008) Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. J Clin Oncol 26(19):3138–3146
14. Adelstein DJ, Saxton JP, Lavertu P, Rybicki LA, Esclamado RM, Wood BG, Strome M, Carroll MA (2002) Maximizing local control and organ preservation in stage IV squamous cell head and neck cancer with hyperfractionated radiation and concurrent chemotherapy. J Clin Oncol 20:1405–1410
15. Garden AS, Harris J, Vokes EE, Forastiere AA, Ridge JA, Jones C, Horwitz EM, Glisson BS, Nabell L, Cooper JS, Demas W, Gore E (2004) Preliminary results of radiation therapy oncology group 9703: a randomized phase ii trial of concurrent radiation and

chemotherapy for advanced squamous cell carcinomas of the head and neck. J Clin Oncol 22(14):2856–2864
16. Denis F, Garaud P, Bardet E, Alfonsi M, Sire C, Germain T, Bergerot P, Rhein B, Tortochaux J, Calais G (2004 Jan 1) Final results of the 94-01 French head and neck oncology and radiotherapy group randomized trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. Clin Oncol 22(1):69–76 Epub 2003 Dec 2
17. Machtay M, Moughan J, Trotti A, Garden AS, Weber RS, Cooper JS, Forastiere A, Ang KK (2008) Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. J Clin Oncol 26:3582–3589
18. Hrzenjak A, Moinfar F, Kremser ML, Strohmeier B, Staber PB, Zatloukal K, Denk H (2006) Valproate inhibition of histone deacetylase 2 affects differentiation and decreases proliferation of endometrial stromal sarcoma cells. Mol Cancer Ther 5:2203–2210
19. Huang BH, Laban M, Leung CH et al (2005) Inhibition of histone deacetylase 2 increases apoptosis and p21Cip1/WAF1 expression, independent of histone deacetylase 1. Cell Death Differ 12:395–404
20. Westendorf JJ, Zaidi SK, Cascino JE, Kahler R, van Wijnen AJ, Lian JB, Yoshida M, Stein GS, Li X (2002) Runx2 (Cbfa1, AML-3) interacts with histone deacetylase 6 and represses the p21(CIP1/WAF1) promoter. Mol Cell Biol 22:7982–7992
21. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Pérez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MÁ, Ahn N, Imhof A, Caldas C, Jenuwein T, Esteller M (2005) Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 37:391–400
22. Park SY, Jun JA, Jeong KJ et al (2011) Histone deacetylases 1, 6 and 8 are critical for invasion in breast cancer. Oncol Rep 25:1677–1168
23. Senese S, Zaragoza K, Minardi S et al (2007) Role for histone deacetylase 1 in human tumor cell proliferation. Mol Cell Biol 27: 47844795
24. Oehme I, Deubzer HE, Wegener D, Pickert D, Linke JP, Hero B, Kopp-Schneider A, Westermann F, Ulrich SM, von Deimling A, Fischer M, Witt O (2009) Histone deacetylase 8 in neuroblastoma tumorigenesis. Clin Cancer Res 15(1):91–99
25. Haberland M, Montgomery RL, Olson EN (2009) The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 10:32–42
26. Peng L, Seto E (2011) Deacetylation of nonhistone proteins by HDACs and the implications in cancer. Handb Exp Pharmacol 206:39–56
27. Marks P, Rifkind RA, Richon VM et al (2001) Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 1:194–202
28. Dokmanovic M, Marks PA (2005) Prospects: histone deacetylase inhibitors. J Cell Biochem 96:293–304
29. Takumi S, Katsuhiro U, Takeshi O et al (2006) Aberrant expression of histone deacetylase 6 in oral squamous cell carcinoma. Int J Oncol 29:117–124
30. Weidle UH, Grossmann A (2000) Inhibition of histone deacetylases: a new strategy to target epigenetic modifications for anticancer treatment. Anticancer Res 20:1471–1485
31. Rothgiesser KM, Fey M, Hottiger MO (2010) Acetylation of p65 at lysine 314 is important for late NF-κB-dependent gene expression. BMC Genomics 11:22
32. Buerki C, Rothgiesser KM, Valovka T, Owen HR, Rehrauer H, Fey M, Lane WS, Hottiger MO (2008) Functional relevance of novel p300-mediated lysine 314 and 315 acetylation of RelA/p65. Nucleic Acids Res 36(5):1665–1680
33. Sterner DE, Berger SL (2000) Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev 64:435–459
34. Rothgiesser KM, Erener S, Waibel S, Luscher B, Hottiger MO (2010) SIRT2 regulates NF-κB dependent gene expression through deacetylation of p65 Lys310. J Cell Sci 123:4251–4258
35. Lehrmann H, Pritchard LL, Harel-Bellan A (2002) Histone acetyltransferases and deacetylases in the control of cell proliferation and differentiation. Adv Cancer Res 86:41–65
36. Gregoretti IV, Lee YM, Goodson HV (2004) Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. J Mol Biol 338(1):17–31
37. Khan O, La Thangue NB (2012) HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications. Immunol Cell Biol 90:85–94
38. Kim HJ, Bae SC (2011) Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anticancer drugs. Am J Transl Res 3(2):166–179
39. Johnstone RW, Licht JD (2003) Histone deacetylase inhibitors in cancer therapy: is transcription the primary target? Cancer Cell 4(1): 13–18
40. West AC, Johnstone RW (2014) New and emerging HDAC inhibitors for cancer treatment. J Clin Invest 124:30–39
41. Blumenschein GR, Kies MS, Papadimitrakopoulou VA et al Phase II trial of the histone deacetylase inhibitor vorinostat (Zolina°, SAHA) in patients with recurrent and/or metastatic head and neck cancer. Investig New Drugs 26(1):81–87
42. Gillenwater AM, Zhong M, Lotan R (2007) Histone deacetylase inhibitor suberoylanilide hydroxamic acid induces apoptosis through both mitochondrial and Fas (Cd95) signaling in head and neck squamous carcinoma cells. Mol Cancer Ther 6(11):2967–2975
43. Takada Y, Gillenwater A, Ichikawa H, Aggarwal BB (2006) Suberoylanilide hydroxamic acid potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis by suppressing nuclear factor-{kappa}B activation. J Biol Chem 281:5612–5622
44. Bali P, Pranpat M, Swaby R, Fiskus W, Yamaguchi H, Balasis M, Rocha K, Wang HG, Richon V, Bhalla K (2005) Activity of suberoylanilide hydroxamic acid against human breast cancer cells with amplification of her-2. Clin Cancer Res 11(17): 6382–6389
45. Kelly WK, O'Connor OA, Krug LM et al (2005) Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol 23:3923–3931
46. Kumar B, Yadav A, Lang JC et al (2015) Suberoylanilide hydroxamic acid (SAHA) reverses chemoresistance in head and neck cancer cells by targeting cancer stem cells via the downregulation of nanog. Genes and Cancer 6(3):169–168
47. Tsai LL, Yu CC, Chang YC et al (2014) Markedly increased Oct4 and Nanog expression correlates with cisplatin resistance in oral squamous cell carcinoma. J Oral Pathol Med 40:621628
48. Lu X, Mazur SJ, Lin T, Appella E, Xu Y (2014) The pluripotency factor nanog promotes breast cancer tumorigenesis and metastasis. Oncogene 33:2655–2664
49. Chung YL, Wang AJ, Yao LF (2004) Antitumor histone deacetylase inhibitors suppress cutaneous radiation syndrome: implications for increasing therapeutic gain in cancer radiotherapy. Mol Cancer Ther 3:317–325
50. Christiansen AJ, West A, Banks KM, Haynes NM, Teng MW, Smyth MJ, Johnstone RW (2011) Eradication of solid tumors using histone deacetylase inhibitors combined with immune-stimulating antibodies. Proc Natl Acad Sci U S A 108:4141–4146
51. Manning J, Indrova M, Lubyova B, Pribylova H, Bieblova J, Hejnar J, Simova J, Jandlova T, Bubenik J, Reinis M (2008) Induction of MHC class I molecule cell surface expression and epigenetic activation of antigen-processing machinery components in a murine model for human papilloma virus 16-associated tumors. Immunology 123:218–227

52. Bourhis J, LeMaitre A, Baujat B et al (2007) Individual patients' data meta-analyses in head and neck cancer. Curr Opin Oncol 19: 188–194
53. Murphy BA, Gilbert J, Cmelak A, Ridner SH (2007) Symptom control issues and supportive care of patients with head and neck cancers. Clin Adv Hematol Oncol 5(10):807–822
54. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, Komaroff E, Nalysnyk L, Zilberberg MD (2003) Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. Radiother Oncol 66:253–262
55. Maesschalck T, Dulguerov N, Caparrotti F et al (2016) Comparison of the incidence of osteoradionecrosis with conventional radiotherapy and intensity-modulated radiotherapy. Head Neck 38:1695–1702
56. Bishop S, Reed WM (2015) The provision of enteral nutritional support during definitive chemoradiotherapy in head and neck cancer patients. J Med Radiat Sci 62:267–276
57. Hutcheson KA, Lewin JS, Barringer DA, Lisec A, Gunn GB, Moore MWS, Holsinger FC (2012) Late dysphagia after radiotherapy-based treatment of head and neck cancer. Cancer 118:5793–5799
58. Ang KK, Zhang Q, Rosenthal DI, Nguyen-Tan PF, Sherman EJ, Weber RS, Galvin JM, Bonner JA, Harris J, el-Naggar AK, Gillison ML, Jordan RC, Konski AA, Thorstad WL, Trotti A, Beitler JJ, Garden AS, Spanos WJ, Yom SS, Axelrod RS (2014) Randomized phase III trial of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III to IV head and neck carcinoma: RTOG 0522. J Clin Oncol 32:2940–2950
59. Ramalingam SS, Parise RA, Ramanathan RK et al (2007) Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. Clin Cancer Res 13:3605–3610
60. Haigentz M Jr, Kim M, Sarta C, Lin J, Keresztes RS, Culliney B, Gaba AG, Smith RV, Shapiro GI, Chirieac LR, Mariadason JM, Belbin TJ, Greally JM, Wright JJ, Haddad RI (2012) Phase II trial of the histone deacetylase inhibitor romidepsin in patients with recurrent/metastatic head and neck cancer. Oral Oncol 48:1281–1288
61. Caponigro F, Di Gennaro E, Ionna F et al (2016) Phase II clinical study of valproic acid plus cisplatin and cetuximab in recurrent and/or metastatic squamous cell carcinoma of head and neck-V-CHANCE trial. BMC Cancer 16(1):918
62. Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F (2003) Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 63:7291–7300
63. Workman JL, Kingston RE (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem 67:545–579
64. Sato T, Suzuki M, Sato Y, Echigo S, Rikiishi H (2006) Sequence-dependent interaction between cisplatin and histone deacetylase inhibitors in human oral squamous cell carcinoma cells. Int J Oncol 28:1233–1241
65. Gressette M, Vérillaud B, Jimenez-Pailhès AS, Lelièvre H, Lo KW, Ferrand FR, Gattolliat CH, Jacquet-Bescond A, Kraus-Berthier L, Depil S, Busson P (2014) Treatment of nasopharyngeal carcinoma cells with the histone-deacetylase inhibitor abexinostat: cooperative effects with cis-platin and radiotherapy on patient-derived xenografts. PLoS One 9(3):e91325
66. Jung M, Kozikowski A, Dritschilo A (2005) Rational design and development of radiation-sensitizing histone deacetylase inhibitors. Chem Biodivers 2:1452–1461
67. Chinnaiyan P, Vallabhaneni G, Armstrong E, Huang SM, Harari PM (2005) Modulation of radiation response by histone deacetylase inhibition. Int J Radiat Oncol Biol Phys 62(1):223–229
68. Chen X, Wong P, Radany EH, Stark JM, Laulier C, Wong JYC (2012) Suberoylanilide hydroxamic acid as a radiosensitizer through modulation of RAD51 protein and inhibition of homology-directed repair in multiple myeloma. Mol Cancer Res 10(8):1052–1064
69. Blattmann C, Oertel S, Ehemann V, Thiemann M, Huber PE, Bischof M, Witt O, Deubzer HE, Kulozik AE, Debus J, Weber KJ (2010) Enhancement of radiation response in osteosarcoma and rhabdomyosarcoma cell lines by histone deacetylase inhibition. Int J Radiat Oncol Biol Phys 78(1):237–245
70. Chen X, Wong P, Radany E, Wong JYC (2009) HDAC inhibitor, valproic acid, induces p53–dependent radiosensitization of colon cancer cells. Cancer Biother Radiopharm 24(6):689–699
71. Losson H, Schnekenburger M, Dicato M, et al (2016) Natural compound histone deacetylase inhibitors (HDACi): synergy with inflammatory signaling pathway modulators and clinical applications in cancer. Molecules 21(11)
72. Wang D, Zhao M, Chen G, Cheng X, Han X, Lin S, Zhang X, Yu X (2013) The histone deacetylase inhibitor vorinostat prevents TNFα-induced necroptosis by regulating multiple signaling pathways. Apoptosis 18(11):1348–1362
73. Cantley MD, Fairlie DP, Bartold PM, Rainsford KD, le GT, Lucke AJ, Holding CA, Haynes DR (2011) Inhibitors of histone deacetylases in class I and class II suppress human osteoclasts in vitro. J Cell Physiol 226(12):3233–3324
74. Bruzzese F, Leone A, Rocco M, Carbone C, Piro G, Caraglia M, di Gennaro E, Budillon A (2011) HDAC inhibitor vorinostat enhances the antitumor effect of gefitinib in squamous cell carcinoma of head and neck by modulating ErbB receptor expression and reverting EMT. J Cell Physiol 226(9):2378–2390
75. Kral AM, Ozerova N, Close J, Jung J, Chenard M, Fleming J, Haines BB, Harrington P, Maclean J, Miller TA, Secrist P, Wang H, Heidebrecht RW Jr (2014) Divergent kinetics differentiate the mechanism of action of two HDAC inhibitors. Biochemistry 53(4): 725–734
76. Rinkel RN, Verdonck-de Leeuw IM, Doornaert P, Buter J, de Bree R, Langendijk JA, Aaronson NK, Leemans CR (2016) Prevalence of swallowing and speech problems in daily life after chemoradiation for head and neck cancer based on cut-off scores of the patient-reported outcome measures SWAL-QOL and SHI. Eur Arch Otorhinolaryngol 273(7):1849–1855