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Phase I study of vandetanib with radiation therapy with or without cisplatin in locally advanced head and neck squamous cell carcinoma

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Abstract

Background—Vandetanib, added to cisplatin and radiation (RT) overcomes chemo RT and EGFR inhibitor resistance in head and neck squamous cell carcinoma (HNSCC) lines and models.

Methods—Patients with previously untreated HNSCC received vandetanib daily for 14 days (starting dose 100 mg) then vandetanib +RT (2.2 Gy/day, 5 days/week) for 6 weeks (regimen 1) or vandetanib +RT (2 Gy/day, 5 days/week) + cisplatin (30 mg/m^2 weekly) for 7 weeks (regimen 2). Primary objective was the maximum tolerated dose (MTD) of vandetanib with RT +/− cisplatin.

Results—Of 33 treated patients, 30 completed therapy (regimen 1, n=12; regimen 2, n=18). MTD in regimen 2 was 100 mg [3 dose limiting toxicities (DLT) at 200 mg], while regimen 1 was stopped due to poor recruitment (one DLT at 200 mg). Most common grade ≥3 AEs were dysphagia (30%), stomatitis (33%) and mucosal inflammation (27%). Five patients discontinued vandetanib due to AEs.

Conclusions—Vandetanib with chemo RT was feasible.

Keywords

vandetanib; cisplatin; radiation therapy; head and neck squamous cell cancer

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Introduction

Primary head and neck squamous cell carcinoma (HNSCC) represents approximately 3% of all newly diagnosed cancers in the USA[1] and is the sixth most common cancer worldwide.[1] HNSCC encompasses squamous cell carcinomas arising from the aerodigestive tract affecting the oral cavity, oropharynx, pharynx and larynx[2]. At diagnosis, a significant proportion of patients (>60%) present with local-regionally advanced stage III-IV HNSCC.[3] Many patients with oropharyngeal squamous cell carcinomas do not have any of the standard risk factors associated with head and neck cancers (e.g., smoking, smokeless tobacco, alcohol consumption). Epidemiologic and molecular studies have identified the HPV-16 genotype of human papillomavirus (HPV) as a causative agent in the majority of these patients[4]. Multiple clinical studies have demonstrated that the prognosis for patients with HPV associated oropharyngeal cancer is significantly better than for those with HPV negative cancer of a comparable stage[5-8]. HNSCC that arise from habitual exposure to carcinogens have lower cure rates than HPV positive tumors with 5-year survival rates between 20%–50% for stage III, and declining to 10%–30% at stage IV.[9] Discouragingly, For the HPV negative HNSCC, survival rates have remained largely unchanged in the last three decades. [10]

Concomitant platinum-based chemoradiotherapy is the current standard of care for patients with locally advanced HNSCC. Despite an 8% improvement in a 5-year survival rate with concurrent chemoradiotherapy regimens,[11] drug-related toxicity and high risk of locoregional recurrence present key barriers to effective treatment.[12-14] For example, nephro- and neurotoxic effects, nausea, vomiting and severe mucositis have all been ascribed to a standard regimen of 3-weekly cisplatin 100 mg/m^2 and ~70 Gy radiation, which limits the benefits of this treatment to patients with normal creatinine clearance and good performance status.[14] While different chemoradiation administration schedules have been explored in efforts to improve survival and organ preservation without increases in toxicity, attention has also focused towards the investigation of multimodal regimens incorporating agents targeting signalling pathways commonly altered in HNSCC.[15,16]

The epidermal growth factor receptor (EGFR), a member of the ErbB receptor family, is frequently overexpressed in HNSCC and has been reported to be a significant prognostic biomarker.[17,18] As a key mediator of tumor angiogenesis, vascular endothelial growth factor receptor (VEGFR) levels have also been subject to investigation in HNSCC.[19-21] Studies generally note elevated expression of VEGFR in HNSCC tumors with some prognostic association, although results are somewhat unclear in this regard.[19-21] More consistent evidence supports the involvement of EGFR and VEGFR up-regulation in mediating radiation resistance in endothelial cells. [22-24]

Vandetanib is a once-daily, oral anti-cancer agent that selectively targets VEGFR, EGFR and REarranged during Transfection (RET) tyrosine kinases.[25,26] Preclinical evidence supports the potential for clinical activity with vandetanib in HNSCC.[27,28] In an established animal model, the administration of vandetanib at a clinically relevant dose was shown to enhance the antitumor effects of radiation therapy (RT) by inhibition of both EGFR and VEGFR signalling in HNSCC human tumor xenografts.[27] Response in EGFR-driven tumors

was evident regardless of whether vandetanib was administered concomitantly or sequentially with RT. In both *in vitro* and *in vivo* models of HNSCC, the addition of vandetanib to combination therapy with cisplatin and RT ameliorated chemoradiation resistance, as demonstrated by prolonged cell survival, decreased cervical lymph node metastases and increased tumor endothelial cell apoptosis. [28]

This Phase I study aimed to determine the maximum tolerated dose (MTD), safety and tolerability of vandetanib in combination with RT with or without cisplatin chemotherapy in patients with previously untreated, unresected, locally advanced (stage III–IV) HNSCC.

Patients and methods

Patients

Eligible patients were aged ≥18 years with histologically or cytologically confirmed squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx or larynx that was not previously treated or resected (stage III–IV with no proven haematogenous metastatic disease). Patients were included if they had a World Health Organization (WHO) performance status (PS) 0–1, life expectancy ≥12 weeks and normal cardiac, haematopoietic, hepatic and renal function. Patients with a history of non-melanoma skin cancer, or other previous malignancies treated ≥3 years prior to current tumor from which the patient had remained continually disease-free and patients with *in situ* carcinoma of the cervix and adequately treated basal cell or squamous cell carcinoma of the skin were eligible for inclusion.

Patients were excluded if they had received any previous anti-cancer therapy for treatment of their current diagnosis, other investigational drugs within the past 30 days or any concomitant medications that may affect QTc or induce CYP3A4 function and could not be discontinued. Other exclusion criteria included the presence of simultaneous primary tumors or major surgery in the 4 weeks prior to screening, or an incompletely healed surgical incision. For regimen 2, additional exclusion criteria included pre-existing neuropathy CTCAE grade ≥2, known severe hypersensitivity to cisplatin and evidence of pre-existing moderate to severe decline of hearing capacity. The study was reviewed and approved by all local institutional review boards ans all patients provided written informed consent.

Study design and treatment

This was an open-label, non-comparative, multicenter, Phase I trial (clinical trials.gov NCT00450138). The study was approved by the responsible ethics committees at each study site, and was conducted according to the principles of the Declaration of Helsinki, International Conference on Harmonisation Good Clinical Practice guidelines and the AstraZeneca policy on bioethics. [29]

Patients were assigned to once-daily vandetanib for 14 days followed by treatment with one of two treatment regimens based on their disease stage according to the American Joint Committee on Cancer Staging (6th edition)[30]: regimen 1, vandetanib in combination with radiotherapy (2.2 Grays [Gy]/day accelerated fraction, 5 days/week) for 6 weeks or regimen 2, vandetanib in combination with radiotherapy (2 Gy/day, 5 days/week) and cisplatin (30

mg/m^2, 2-hour intravenous infusion/week) for 7 weeks. The choice of 30 mg/m^2 versus 40 mg was based on the potential for increased hematologic toxicity with the combination. Patients with disease stages T1N1 and T2N1 were only assigned to regimen 1 while those with T2N2b, T2N2c, T2N3, T3 and T4 (any N) were only assigned to regimen 2. Patients with a disease stage of T1N2a, T1N2b, T1N2c or T2N2a were eligible for either regimen. Patients of an eligible stage for regimen 2, who were medically not appropriate candidates for chemotherapy, could be enrolled in regimen 1 at the investigator's discretion.

A standard dose-escalation design was adopted for this study. In each regimen, a minimum of six patients was initially enrolled, and vandetanib treatment was started at a dose of 100 mg/day. Dose escalation was based on toxicity information collected during treatment and a 30-day follow-up period, and the decision to proceed with dose escalation was determined by a safety review committee. If <2 of 6 evaluable patients experienced a dose-limiting toxicity (DLT), the dose was defined as tolerable and was escalated to the next dose level. If ≥2 of 6 evaluable patients experienced a DLT, this dose was considered the non-tolerated dose (NTD). Enrolment into this dose was stopped immediately, with no dose escalation. The dose preceding the NTD was therefore considered to be the MTD. A DLT had to be attributable to the study treatment (vandetanib/radiotherapy/cisplatin) in the opinion of the investigator and consisted of any of the following: non-hematologic toxicity grade ≥3 despite optimal symptomatic care; hematologic grade 4 toxicity (excluding neutropenia if duration <8 days); grade 3 thrombocytopenia associated with bleeding (not applicable to patients on therapeutic anticoagulation); QTc prolongation (a single measurement ≥550 ms or an increase of ≥100 ms from baseline; two consecutive measurements ≥500 ms but <550 ms; or an increase of ≥60 ms, but <100 ms, from baseline to ≥480 ms using Bazett's correction factor); during RT treatment, any of the following grade 4 toxicities: dysphagia, mucositis/stomatitis, skin toxicity, xerostomia and hypogeusia; or any delay in RT >5 days due to toxicity. All patients maintained pill diaries that were collected by the research nurses.

Treatment was continued for 8–9 weeks unless patients experienced a DLT or met another criterion for discontinuation. After treatment completion, patients entered a 30-day follow-up phase of the study, during which safety, response, survival and relapse were evaluated; safety was also evaluated at 60 days post-treatment completion. Patients were subsequently monitored every three months until a total of two years' follow-up was achieved, evaluating response, survival and relapse.

The primary objective was to establish the MTD, safety and tolerability of vandetanib in combination with radiotherapy, or weekly cisplatin and radiotherapy. Secondary objectives included objective tumor response rate (ORR), disease control rate (DCR) and locoregional control rate (LCR), locoregional recurrence and distant disease recurrence at 2 years, PFS, duration of locoregional control, and pharmacokinetics (PK) of vandetanib and cisplatin following combined treatment. Exploratory objectives included correlations between biomarker levels and efficacy and safety of vandetanib in combination with radiotherapy or chemoradiotherapy.

Assessments

Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCICTCAE, version 3.0). Additional safety measures included physical examination, ECG, vital signs and laboratory findings. Tumor assessments were performed according to Response Evaluation Criteria In Solid Tumors (RECIST; version 1.0),[31] and were measured at baseline, 30 and 90 days after treatment completion and every 3 months during follow-up. ORR was defined as complete response (CR) plus partial response (PR). Locoregional control was defined according to disease control rate (CR plus PR plus stable disease [SD] >12 weeks), but excluding distant disease. Progression-free survival (PFS) was calculated from the date of first dose of vandetanib until the date of objective disease progression (locoregional or distant) or death (by any cause in the absence of progression), provided death was within 3 months from the last evaluable RECIST assessment. The duration of locoregional control was defined as the time from first dose of treatment until the date of the first PFS event, where progression was determined using only lesions in the head and neck and neck lymphatic nodes and was derived for patients with locoregional control. Assessment of PK and biomarkers methodology is included in supplementary materials.

Statistical analysis

No formal sample size calculations were undertaken; on the basis of the traditional dose-escalation design used in the study, it was calculated that approximately 48 patients would be required for study completion. The safety analysis set comprised all patients who received at least one dose of vandetanib. All patients who received ≥1 dose of vandetanib and had evaluable PK data were included in the PK analysis set. Formal statistical comparisons between treatment arms were not performed. Safety data are presented descriptively. Secondary efficacy parameters are presented with, where applicable, 95% confidence intervals (CI; Wilcoxon method). Estimates of PFS distribution were obtained by Kaplan-Meier survival analyses and differences in survival between groups established with the log-rank test. The relationship between changes in CAF biomarkers or baseline tumor markers and PFS was tested using the transformation of logarithm to the base 2 of a respective marker (to satisfy the normality assumption of the linear mixed effect model) and the Cox proportional hazards model.

Results

Patients

A total of 38 patients were enrolled from three centers between December 2006 and September 2009; 33 received treatment with vandetanib 100 or 200 mg/day in combination with radiotherapy (n=12) or cisplatin/radiotherapy (n=21) and comprised the safety analysis set and the efficacy set. Five patients were excluded from safety analysis as they did not receive a single dose of vandetanib. A total of 30 patients were included in the PK analysis set; eight patients were excluded (the five who did not receive a single dose of vandetanib and three patients withdrew from treatment before receiving cisplatin or radiotherapy.

A total of six, two and one patient(s) discontinued vandetanib, cisplatin or radiotherapy, respectively, prior to completing the full course of treatment; the main reason for discontinuation was due to AEs. Overall, 21 patients completed the 2-year follow-up; reasons for discontinuation from the study were progression/recurrence of disease (n=6), voluntary withdrawal (n=2), loss to follow-up (n=2), safety reasons (n=1) and death (n=1). The mean total duration of vandetanib exposure (accounting for dose interruptions) for regimen 1 was 53 days (vandetanib 100 mg) and 54.7 days (vandetanib 200 mg) and for regimen 2 was 61.8 days (vandetanib 100 mg) and 50.8 days (vandetanib 200 mg).

Patients had a mean age of 55 years, and were predominantly male (n=28/33) and Caucasian (n=32/33; Table 1). In both regimens, the oropharynx was the most common primary tumor site and the majority of patients had a WHO PS of 0 (n=27/33) and IVA stage of disease (n=26/33). Among the vandetanib plus radiotherapy groups, the majority of patients had a TNM classification of T1 (n=11/12), while in the vandetanib and cisplatin/radiotherapy groups the majority had T2 (n=7/21) or T3 (n=12/21) tumors. Of 27 patients with p16 tumor status available, 24 (89%) had p16 positive tumors.

Safety and tolerability

Overall, four patients experienced DLTs. With regimen 1, one DLT (grade 3 ALT elevation) occurred in the vandetanib 200 mg cohort; further dose escalation was not performed because of low patient recruitment. Subsequently, this treatment regimen was stopped and the MTD for regimen 1 was therefore not determined. With regimen 2, at a vandetanib dose level of 200 mg, DLTs were reported in 3 of 6 patients (grade 4 pulmonary embolism and recurrent grade 3 diarrhea; grade 3 elevated creatinine and grade 5 acute renal failure, acute respiratory distress syndrome and pneumonia with normal ANC; grade 3 neutropenic fever, prolonged QTc, and *colitis-pneumatosis* and grade 4 events of low platelets, low leukocytes and non-ST elevation myocardial infarctions). Vandetanib 200 mg in combination with cisplatin/radiotherapy was considered not tolerated and vandetanib 100 mg was declared the MTD; this cohort was expanded to include an additional nine patients.

The most commonly experienced AEs were radiation skin injury (n=29; 87.9%), fatigue (n=28; 84.8%) and constipation (n=27; 81.8%; Table 2). The most frequent AEs of grade ≥3 that occurred with both treatment regimens were stomatitis (n=11; 33.3%), dysphagia (n=10; 30.3%) and mucosal inflammation (n=9; 27.3%; Table 2). Grade 3 AEs commonly experienced by patients treated in regimen 2, but not reported for regimen 1, were diarrhea (n=4/21), dehydration (n=5/21) and hyponatremia (n=5/21).

Serious AEs (SAEs) were experienced by seven patients in total: one patient in regimen 1 (vandetanib 200 mg dose level) and six patients in regimen 2 (three patients each at the vandetanib 100 mg and 200 mg dose levels). The most common SAE was diarrhea, experienced by one patient in regimen 1 and three patients in regimen 2 (vandetanib 200 mg). One patient in regimen 2 (vandetanib 200 mg) experienced SAEs that subsequently led to death (acute renal failure, acute respiratory distress syndrome and pneumonia). This event was considered by the investigator to be causally related to cisplatin.

A total of five patients experienced AEs that led to discontinuation of vandetanib. In regimen 1, one patient (vandetanib 200 mg) discontinued due to grade 3 chills and grade 2 diarrhea; in regimen 2, one patient receiving vandetanib 100 mg discontinued due to grade 1 elevated blood creatinine levels, and three patients receiving vandetanib 200 mg discontinued due to grade 3 skin disorder (n=1); grade 4 pulmonary disorder and grade 3 diarrhea (n=1); grade 5 renal failure, pneumonia, and acute respiratory distress and grade 3 elevated blood creatinine levels, as mentioned above under SAEs and ascribed to cisplatin(n=1). Two patients experienced AEs that led to dose interruption of vandetanib: one patient in regimen 1 (vandetanib 100 mg; pruritus and maculopapular rash) and one patient in regimen 2 (vandetanib 200 mg; diarrhea). There were no AE-related dose reductions of vandetanib in any treatment group.

Clinical laboratory assessments revealed that one patient in regimen 1 (vandetanib 200 mg) experienced grade 3 ALT elevation; no other laboratory abnormalities of grade ≥3 were reported with this regimen. For regimen 2, three patients experienced decreased leukocyte count (vandetanib 100 mg n=2; 200 mg n=1) and one patient (vandetanib 200 mg) experienced elevated International Normalized Ratio levels. Within this regimen, grade 3 abnormalities included hyponatremia (vandetanib 100 mg, n=2; 200 mg, n=2) and hypokalemia (vandetanib 100 mg, n=1). Three patients in regimen 2 (vandetanib 100 mg, n=2; 200 mg, n=1) experienced an AE of QT prolongation. However, no patient had a QTc of ≥500 ms and none met the protocol-defined criteria for QT prolongation. There were no clinically significant changes in vital signs during this study.

Efficacy

Response was assessed at 3 months after completion of therapy. Twenty-eight of 33 patients evaluable for efficacy achieved complete response (CR) and one patient achieved a partial response (PR) and the majority of patients achieved disease and locoregional control (Table 3). Within the evaluable patient population (29patients), CR rate was 92% (11/12) for regimen 1 and 100% (17/17). Twenty-four of 28 patients with a best objective response of CR completed the predefined study follow-up of 2 years without disease recurrence; RECIST-confirmed locoregional recurrence occurred in 4patients, receiving all regimen 2 (vandetanib 100 mg), and one death occurred during treatment ascribed to cisplatin toxicity in regimen 2 (vandetanib 200 mg), while a patient in regimen 1 (vandetanib 200 mg) who did not have confirmation of CR with 2 consecutive CT scan assessments developed new lesions at 9 months follow-up.. At data cut-off (2 December 2011), six progression events had occurred and with the 2 year PFS as an endpoint (Table 3), leading to progression rates ranging between 0% (0/6 evaluable patients) in regimen 1 (vandetanib 100 mg) and 27% (4/15 evaluable patients) in regimen 2 (vandetanib 100 mg). Additional progression events (regimen 2 at 100 mg of vandetanib) not included in Table 3 include a patient who experienced biopsy-proven recurrence although imaging findings did not reveal it and a second patient progressing with distant metastasis that occurred beyond the pre-specified 24 month follow-up period. Overall 2 patients experienced distant recurrence, while for the remaining patients with local/regional recurrence underwent salvage surgery. Over a median follow-up of 19.8 months, the median PFS (see Table 6 in supplementary materials for correlation of PFS with clinicopathological and biomarker data, based on a total of 8 events)

and duration of locoregional control was not reached for any treatment regimen. The one year overall survival, PFS and locoregional control rates were all 96.9% (95% CI 91, 100).

Of 25 evaluable patients with available HPV status, 23 tested positive for HPV (regimen 1, vandetanib 100 mg n=6, 200mg n=5; regimen 2, vandetanib 100 mg n=8, 200 mg n=4). All patients achieved CR regardless of baseline HPV status. Twenty-one of 23 HPV-positive patients and one of two patients with HPV-negative status had not experienced locoregional recurrence by study completion. RECIST-confirmed locoregional recurrence was noted in 2/8 HPV-positive patients (25%) on regimen 2 vandetanib 200 mg.

Pharmacokinetics

Following multiple dosing, vandetanib demonstrated dose-related increases in exposure (Table 4). Mean plasma concentrations of vandetanib were higher at the vandetanib 200 mg dose level than the 100 mg dose level following repeat dosing (Day 15 and 50) and were higher at Week 8 than Week 3 due to increased exposure (Supplementary Figure 1). This indicates accumulation of vandetanib over the treatment period, due to the long half-life of vandetanib.

Overall, administration of radiotherapy with or without cisplatin during once-daily dosing of vandetanib 100 mg or 200 mg did not appear to affect the steady-state exposure to vandetanib. There was no evidence of a difference in cisplatin exposure (C_{max} or $AUC_{0–24}$) between background therapy of vandetanib 100 mg or 200 mg. On background therapy of vandetanib 100 mg and 200 mg, cisplatin exposure was higher on Day 50 after repeat dosing than on Day 15 (first day of cisplatin dosing), suggesting that there was some accumulation of cisplatin following multiple dosing.

Exploratory analysis by clinical and biomarker status

Plasma biomarker data were available for 31 patients at baseline. High baseline plasma levels of hepatocyte growth factor (HGF), interleukin (IL)-8, macrophage migration inhibitory factor (MIF) and eotaxin were associated with shorter PFS.

Overall, 27 patients had baseline tumor samples evaluable for tissue IHC and subsequent correlation analyses with clinical outcome (Table 6, supplementary materials). When examined according to patient and tumor characteristics, higher nodal stage (≥N2b), lower T stage (<T3), oropharyngeal primary tumors and non-smoking status were all significantly associated with p16-positive tumor status ($P<0.05$). Moreover, the mesenchymal cell marker, vimentin was most frequently expressed in non-smokers. In correlation analyses between patient clinical and pathological characteristics and disease progression, expression of p16 in tumors demonstrated a significant association with superior PFS (HR 0.08 [95% CI 0.01, 0.57]; $P=0.01$).

Discussion

This Phase I dose-escalation study is the first to evaluate dual targeting of VEGFR/EGFR tyrosine kinases with radiotherapy or chemoradiotherapy in patients with HNSCC. Using a standard dose escalation study design, vandetanib 100 mg was identified as the MTD when

administered with radiotherapy and cisplatin. Additionally, vandetanib at doses of 100 and 200 mg was also found to be well tolerated in combination with radiotherapy, although the MTD could not be determined because of premature closure of this regimen due to low patient recruitment. Therefore the primary endpoint was not fully met for this regimen.

The safety profile of vandetanib observed in this study was consistent with previous findings across other cancer types.[32,33] AEs of diarrhea with vandetanib combination treatment in this study and in previous reports likely reflect an effect of EGFR inhibition, as events of diarrhea are also widely observed in patients treated with erlotinib, gefitinib and cetuximab.[32,33] The possibility exists that other AEs experienced by patients in the combination treatment groups, while less commonly reported with vandetanib, occurred as a consequence of cisplatin and/or radiotherapy. Concurrent administration of cisplatin with radiotherapy is associated with a distinct safety profile in HNSCC, primarily involving mucosal toxicity and AEs of dysphagia and dehydration, and one which may necessitate interruptions to drug regimens, with subsequent adverse implications for prognosis.[14,34-37] In agreement with these previous studies, stomatitis, dysphagia and mucosal inflammation were the most frequent all-cause AEs reported here. However, AEs did not frequently lead to dose interruptions (n=2) in this study; of further note, no patient required a reduction of vandetanib dose during combination treatment. Based on lack of survival benefits seen at 5 years with high dose cisplatin in RTOG 91-11[36] we designed the study to offer a weekly cisplatin regimen in an effort to reduce considerable toxicity seen with a 3-week regimen at 100 mg/m^2. Weekly cisplatin administration concurrent to radiation has been adopted as standard of care in some centers based on similar efficacy with 3-weekly (high dose) cisplatin[38].

Our exploratory efficacy findings are suggestive of an anti-tumor effect with vandetanib in combination with radiotherapy or chemoradiotherapy, and appears to be in general agreement with historical controls.[34,35,39] Locoregional control was achieved by the majority of patients (86.7%) at the MTD in this study, and compares favorably with recent 2- or 3-year evaluations of chemoradiotherapy (78%–87%).[34-36] A focus of considerable research effort in locally advanced disease is the high rate (~80%) of recurrence within 2 years post-intervention.[40,41] Among patients who achieved a locoregional best objective response of CR with vandetanib and radiotherapy or chemoradiotherapy, rates of disease recurrence at 2 years were ranged between 0%–30.8% and consistent with historical data for cisplatin-based chemoradiotherapy[36] even though HPV positive cases were included. Nonetheless, the small number of patients precludes definitive conclusions on the efficacy of vandetanib in HNSCC compared with standard chemoradiotherapy. In addition, the non-randomized study design and inclusion of vandetanib in all treatment groups excludes formal comparisons with standard treatment.

There was no evidence that co-administration of cisplatin increased exposure to vandetanib in the pharmacokinetic analyses conducted herein. This result concurs with the absence of a common underlying clearance mechanism by which either agent could alter exposure through metabolic enzyme competition, induction or inhibition. The area under the plasma concentration–time curve at steady state (AUCss) identified in this study was compared with vandetanib 100 mg monotherapy data from previous studies in patients with colorectal

cancer and found to be relatively higher than historical data (7130 ng. H/ml; n=24), but within the same range.[42-44]

Analyses were undertaken to explore the potential prognostic significance of plasma and tumor biomarkers during vandetanib combination treatment. The prognostic role of *EGFR* expression in HNSCC has been previously reported,[17,18] with one sub-analysis from a randomized clinical trial (N=155) highlighting a significant association ($P \leq 0.002$) between enhanced *EGFR* levels and decreased survival in patients with advanced HNSCC enrolled within the radiotherapy arm of the study.[18] In contrast, no correlation between *EGFR* overexpression and clinical outcome was identified in this study.[17,18] Similar observations have been noted with gefitinib, a small-molecule tyrosine kinase inhibitor that bears some similarity of action with vandetanib. A study of 31 patients with locally advanced HNSCC failed to identify an association between *EGFR* expression and response or survival outcome following treatment with gefitinib in combination with cisplatin and radiotherapy.[45] The method of quantifying *EGFR* expression may itself influence potential associations with prognosis. In one study, quantification of protein expression using tissue microarray (A QUA) was observed to predict for clinical outcome in HNSCC patients treated with chemoradiation whereas no such prognostic association was observed according to *EGFR* FISH status.[46] HGF, a hypoxia-induced secreted protein, regulates the expression of the angiogenic factor, IL-8, both of which are implicated in tumor growth and invasion.[46,47] Elevations of HGF and IL-8 at baseline were identified as predictive of adverse survival in the current analyses. The feasibility of HGF and IL-8 as prognostic indicators is given greater credence by comparable results from a recently reported Phase III study of multimodal chemoradiation in locally advanced HNSCC[48] in addition to earlier historical findings.[47] However, small sample sizes in these analyses limit the validity of these conclusions without additional support from studies in larger patient populations.

HPV status has gained widespread recognition as a prognostic factor in HNSCC, especially for tumors arising from the oropharynx.[49-51] In the current study, 21 of 23 baseline HPV-positive patients and one of two HPV-negative patients had no locoregional recurrence of their disease at 2 years, consistent with published evidence indicating an improved outcome for HPV-positive patients although the numbers in this study are small . [49-51]

In conclusion, vandetanib appeared to be generally well tolerated at a dose of 100 mg/day when administered with radiotherapy and cisplatin, however no further development of this regimen in patients with locally advanced HNSCC is planned.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Patient demographics and baseline characteristics

	Regimen 1		Regimen 2		
	Vandetanib 100 mg + RT (n=6)	**Vandetanib 200 mg + RT (n=6)**	**Vandetanib 100 mg + RT + cisplatin (n=15)**	**Vandetanib 200 mg + RT + cisplatin (n=6)**	**All patients n=33**
Mean age, years (range)	50.7 (44–56)	55.3 (51–60)	56.1 (43–71)	56.3 (46–72)	55.0 (43–72)
Male/female, n	5/1	6/0	13/2	4/2	28/5
Race, n					
Caucasian	6	5	15	6	32
Black	–	1	–	–	1
Primary tumor location, n	6	5	10	5	26
Oropharynx	–	–	3	1	4
Supraglottis	–	–	2	–	2
Glottis	–	1	–	–	1
Hypopharynx					
WHO PS, n					
0/1	6/0	6/0	11/4	4/2	27/6
TNM classification					
Primary tumor, n					
T1	6	5	–	–	11
T2	–	1	6	1	8
T3	–	–	8	4	12
T4a	–	–	1	1	2
Regional lymph nodes, n	–	–	3	–	3
N0	1	–	1	1	3
N1	1	–	–	–	1
N2	–	2	–	–	2
N2a	4	4	6	5	19
N2b	–	–	4	–	4
N2c	–	–	1	–	1
N3					
Distant metastases, n	6	6	15	6	33
M0					
Stage of disease, n					
III	1	–	4	1	6
IVA	5	6	10	5	26
IVB	–	–	1	–	2
p16 status					
Neg	0	1	2	0	3
Pos	6	5	8	5	24

Table 2

Most common all-cause adverse events reported in ≥15% of patients overall and grade ≥3 adverse events reported in ≥10% patients overall

All cause AEs	Number of patients with AEs, all grades (CTCAE grade ≥3 number of patients)				
	Regimen 1		Regimen 2		
	Vandetanib 100 mg + RT (n=6)	Vandetanib 200 mg + RT (n=6)	Vandetanib 100 mg + RT + cisplatin (n=15)	Vandetanib 200 mg + RT + cisplatin (n=6)	All patients n=33
Radiation skin injury	6	5 (1)	13 (3)	5	29 (4)
Fatigue	6	4	12	6	28
Constipation	5	5	13	4	27
Oropharyngeal pain	6	5 (2)	11 (4)	4 (2)	26 (8)
Weight decrease	5	5	12	4	26
Dry mouth	6	6	10	3	25
Nausea	6	4	10	5	25
Dysphagia	4 (2)	6 (2)	10 (5)	4 (1)	24 (10)
Diarrhea	4	4	10 (1)	5 (3)	23 (4)
Dysgeusia	6	4	9	3	22
Oral pain	4	3 (1)	9 (3)	5 (2)	21 (6)
Stomatitis	1 (1)	5 (5)	7 (5)	2	15 (11)
Alopecia	4	4	6	1	15
Mucosal inflammation	5 (3)	1 -	4 (3)	3 (3)	13 (9)
Dizziness	2	1	6	3	12
Decreased appetite	4	4	3	1	12
Hypomagnesaemia	3	-	5	4	12
Pyrexia	2	3	4	2	11
Dehydration	1	2	5 (2)	3 (3)	11 (5)
Hyponatremia	-	-	7 (3)	4 (2)	11 (5)
Vomiting	2	2	4	2	10
Cough	-	2	7	-	9
Rash	-	2	6	-	8
Maculo-papular rash	4	-	2	2	8
Insomnia	1	1	4	1	8
Dyspnoea	1	1		3	7
Odynophagia	3	-	3	-	6
Pharyngeal inflammation	-	2 (2)	3 (2)	1 (17)	6 (4)
Pruritus	2	2	1	1	6
Headache	1	1	3	1	6
Hypertension	-	4	1	1	6
Chills	-	2	2	1	5
Hyperhidrosis	2	2	-	1	5
Infection (opportunistic)	1	-	3	1	5

All cause AEs	Number of patients with AEs, all grades (CTCAE grade ≥3 number of patients)				
	Regimen 1		Regimen 2		
	Vandetanib 100 mg + RT (n=6)	Vandetanib 200 mg + RT (n=6)	Vandetanib 100 mg + RT + cisplatin (n=15)	Vandetanib 200 mg + RT + cisplatin (n=6)	All patients n=33
Postoperative wound infection	-	2	2	1	5
Anxiety	-	-	3	2	5
Depression	1	1	3	-	5
Blurred vision	1	-	2	2	5

Table 3

Objective tumor response rate, disease control rate, locoregional control rate and progression rate

	Regimen 1		Regimen 2	
	Vandetanib 100 mg + RT (n=6)	**Vandetanib 200 mg + RT (n=6)**	**Vandetanib 100 mg + RT + cisplatin (n=15)**	**Vandetanib 200 mg + RT + cisplatin (n=6)**
Objective response				
(ITT) Events/N Rate, % (95% CI)	6/6 100.0 (61.0–100.0)	6/6 100.0 (61.0–100.0)	13/15 86.7 (62.1–96.3)	4/6 66.7 (30.0–90.3)
Disease control				
(ITT) Events/N Rate, % (95% CI)	6/6 100.0 (61.0–100.0)	6/6 100.0 (61.0–100.0)	13/15 86.7 (62.1–96.3)	4/6 66.7 (30.0–90.3)
Locoregional cntrl				
(ITT) Events/N Rate, % (95% CI)	6/6 100.0 (61.0–100.0)	6/6 100.0 (61.0–100.0)	13/15 86.7 (62.1–96.3)	4/6 66.7 (30.0–90.3)
Progression[†] Events/N	0/6	1/6	4/15	1/6[*]
Local/Regional Distant		1/6	4/15	

ITT, intent-to treat

[†]At data cut-off (2 December 2011)

[*]death

Table 4

Pharmacokinetic parameters for vandetanib.

	Regimen 1		Regimen 2	
	Vandetanib 100 mg + RT (n=6)	**Vandetanib 200 mg + RT (n=6)**	**Vandetanib 100 mg + RT + cisplatin (n=13)**	**Vandetanib 200 mg + RT + cisplatin (n=3/5 *)**
C_{max} (ng/ml)				
Day 15	331	593	270	589
Day 50	486	1196	5234	749
$AUC_{(0-24)}$(ng.h/ml)				
Day 15	6792	12880	5260	11939
Day 50	10304	25597	10684	15112

* *n=5 for day 15; n=3 for day 50;* C_{max}*, maximum plasma concentration;* $AUC_{(0-24)}$, Area under the plasma concentration-time curve from zero to 24 hours post dose

Table 5

Cytokine and angiogenic factor profiling

CAF category	
Pro/antiangiogenic factors	VEGF, FGF-basic, HGF, PDGF-bb, MMP-9, PlGF, endoglin*
EGF axis CAFs	EGF, EGFR, TGF-α, amphiregulin, betacellulin, HB-EGF
Chemokines	MCP-1, MCP-3, MIP-1α, MIP-1β, RANTES (CCL5), MIP-2, MIG (CXCL-9), eotaxin (CCL11), IP-10 (CXCL10), SDF-1α (CXCR4), KC (CXCL1), GRO-α, CTACK (CCL27)
Interleukins	IL-1α, IL-1β, IL-1RA, IL-2, IL-2Ra, IL3–IL10, IL-12 – IL18
Inflammation/adhesion CAFs	sICAM-1, IFN-α, IFN-γ, TNF-α, TNF-β, MIF, LIF
Hypoxia CAFs	Osteopontin*, CA-9*
Endothelial function/damage CAFs	sVEGFR-2*, sE-selectin, VCAM-1
Growth factors	GM-CSF, G-CSF, M-CSF, SCGF-β, SCF, beta-NGF

* ELISA assays

Table 6

Patient clinical and pathological characteristics by progression status

Covariate	Progression status, n (%)[#]		HR (95% CI)	*P* value[*]
	Progression	Non-progression		
Age				
>55 years	6 (40)	9 (60)	7.3 (0.8, 68.1)	0.08
≤55 years	2 (11.1)	16 (88.9)	1	
Gender				
Female	1 (20)	4 (80)	1.37 (0.16, 11.38)	0.774
Male	7 (25)	21 (75)	1	
Tumor location				
Oropharynx (OPC)	3 (11.5)	23 (88.5)	0.13 (0.03, 0.67)	0.015
Others	5 (71.4)	2 (28.6)	1	
Tumor stage				
T1/T2	3 (15.8)	16 (84.2)	1	
T3/T4	5 (35.7)	9 (64.3)	1.61 (0.36, 7.22)	0.535
Nodal stage				
≤N2a	4 (50)	4 (50.0)	1	
≥N2b	4 (16)	21 (84)	0.18 (0.04, 0.83)	0.028
Stage of disease				
III	3 (50)	3 (50.0)	1	
IV	5 (18.5)	22 (81.5)	0.29 (0.07, 1.32)	0.109
Smoking history				
Non-smoker	2 (14.3)	12 (85.7)	1	
Smoker	6 (31.6)	13 (68.4)	6.16 (0.74, 51.4)	0.093
Smoking status				
Current	4 (50.0)	4 (50.0)	7.47 (0.83, 67.29)	0.073
Former	1 (10.0)	9 (90.0)	2.52 (0.16, 40.57)	0.515
Never	2 (14.3)	12 (85.7)	1	
p16 status				
Negative	3 (100)	0	1	
Positive	4 (16.7)	20 (83.3)	0.08 (0.01, 0.57)	0.01
EGFR classification[†]				
Negative	5 (27.8)	13 (72.2)	1	
Positive	2 (33.3)	4 (66.7)	1.39 (0.25, 7.59)	0.706
EGFR expression[‡]				
≤ Median	3 (21.4)	11 (78.6)	1	
> Median	4 (30.8)	9 (69.2)	2.26 (0.41, 12.35)	0.348
E-cadherin expression[‡]				
≤ Median	2 (15.4)	11 (84.6)	1	
> Median	5 (38.5)	8 (61.5)	3.79 (0.67, 21.48)	0.133

Covariate	Progression status, n (%)[#]		HR (95% CI)	*P* value[*]
	Progression	Non-progression		
Vimentin classification[‡]				
Negative	1 (25.0)	3 (75.0)	1	
Positive	6 (28.6)	15 (71.4)	1.32 (0.15, 11.33)	0.802

[#]Eight events are included in this analysis, 6 as reported in the main manuscript and 2 additional events, including one patient with biopsy but not imaging proven recurrence and a patient with distant metastasis occurring beyond the 24 month pre-specified follow-up period

[*]Univariate derived from Cox proportional hazards model

[†]FISH analysis, EGFR status was not determined for three patients who had not progressed

[‡]Immunohistochemical analysis