Phase II trial of everolimus and erlotinib in patients with platinum-resistant recurrent and/or metastatic head and neck squamous cell carcinoma

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Background: Enhanced phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is one of the key adaptive changes accounting for epidermal growth factor receptor (EGFR) inhibitor-resistant growth in head and neck squamous cell carcinoma (HNSCC). We designed a phase II clinical trial of EGFR tyrosine kinase inhibitor (TKI), erlotinib, in association with the mTOR inhibitor, everolimus, based on the hypothesis that the downstream effects of Akt through inhibition of mTOR may enhance the effectiveness of the EGFR-TKI in patients with recurrent/metastatic HNSCC.

Patients and methods: Patients with histologically or cytologically confirmed platinum-resistant HNSCC received everolimus 5 mg and erlotinib 150 mg daily orally until disease progression, intolerable toxicity, investigator or patient decision. Cytokines and angiogenic factors profile, limited mutation analysis and p16 immunohistochemistry status were included in the biomarker analysis.

Results: Of the 35 assessable patients, 3 (8%) achieved partial response at 4 weeks, 1 confirmed at 12 weeks; overall response rate at 12 weeks was 2.8%. Twenty-seven (77%) patients achieved disease stabilization at 4 weeks, 11 (31%) confirmed at 12 weeks. Twelve-week progression-free survival (PFS) was 49%, median PFS 11.9 weeks and median overall survival (OS) 10.25 months. High neutrophil gelatinase lipocalin (P = 0.01) and vascular endothelial growth factor (VEGF) (P = 0.04) plasma levels were significantly associated with worse OS.

Conclusions: The combination of erlotinib and everolimus did not show significant benefit in unselected patients with platinum-resistant metastatic HNSCC despite a manageable toxicity profile. Markers of tumor invasion and hypoxia identify a group of patients with particularly poor prognosis.

Clinical trial number: NCT00942734.

Key words: HNSCC, mTOR inhibitors, EGFR inhibitors, everolimus, erlotinib

introduction

Head and neck squamous cell carcinoma (HNSCC) is characterized by epidermal growth factor receptor (EGFR) overexpression [1], which is an established negative prognostic factor [2]. The chimeric anti-EGFR monoclonal antibody cetuximab was the first molecularly targeted therapy to receive US Food and Drug Administration approval for the treatment of HNSCC and has since been integrated into the standard of care with limited success in the metastatic setting [1, 3]. Published phase II data support tolerability and antitumor activity for the EGFR tyrosine kinase inhibitor (TKI) erlotinib in HNSCC in recurrent or metastatic disease [1]. Predictive biomarkers for EGFR inhibitor therapy in HNSCC are not clearly defined and EGFR sensitizing mutations observed in non-small-cell lung cancer (NSCLC) are very uncommon in HNSCC.

Published data suggest that the phosphoinositide 3-kinase (PI3K)/Akt pathway may play a central role in predicting response and resistance to EGFR inhibitors in NSCLC [4] and that an enhanced PI3K/Akt/mammalian target of rapamycin (mTOR) pathway may be one of the key adaptive changes accounting for EGFR inhibitor-resistant growth in HNSCC [5]. The mTOR is mainly activated via the PI3K pathway through AKT/protein kinase B and the tuberous sclerosis complex (TSC1/2). Mutations in these components or in phosphatase and tensin homolog (PTEN), a negative regulator of PI3K, may result in their dysregulation. In tongue cancer, PTEN loss has negative prognostic indications [6] and blockade of its upstream
kinase PI3K potently inhibits tumor cell growth [7], indicating an important role of the PI3K pathway in HNSCC [6]. Different cell lines including PTEN-deficient epidermoid carcinoma cells are resistant to treatment with EGFR inhibitors [8].

At the cellular and molecular level, RAD001 (everolimus) acts as a signal transduction-selective mTOR inhibitor. Inhibition of this pathway might therefore prevent or delay the onset of resistance to anti-EGFR therapy. The combination of the mTOR inhibitor everolimus and the EGFR-TKI erlotinib has shown a good safety and tolerability profile, with modest activity in advanced NSCLC [9]. We present the results of a phase II clinical trial of mTOR inhibitor, everolimus, and EGFR inhibitor, erlotinib, in patients with recurrent/metastatic HNSCC. The clinical trial design was based on the hypothesis that the ability to inhibit the downstream effects of Akt through inhibition of mTOR will enhance the effectiveness of the EGFR-TKIs in patients with recurrent/metastatic HNSCC. In an effort to identify a cohort of patients who might benefit from the combination therapy, we also analyzed the plasma cytokines and angiogenic factors (CAF) profile from blood samples at different time points (baseline, 4 and 12 weeks), a limited panel of mutations as well as the p16 status of the tumors as a surrogate for human papillomavirus (HPV) infection [10].

materials and methods

patients

Major inclusion criteria included: histologically or cytologically confirmed HNSCC after failing one platinum-containing chemotherapy with or without EGFR-TKI; age ≥18 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; measurable disease according to response evaluation criteria in solid tumors (RECIST) [11]; adequate cardiac, hematopoietic, renal and hepatic function. Prior investigational therapy was allowed with wash-out period of at least 4 weeks with the exclusion of mTOR inhibitors. A minimum of 2 weeks since major surgery or completion of radiation therapy was allowed. Major exclusion criteria were chronic steroid treatment or other immunosuppressive agents unless discontinued 24 h before initiation of study drugs; brain metastatic disease unless treated, controlled and off steroids and/or antiepileptics for at least 3 weeks.

study design and treatment

In this open-label, single-institution, two-stage, phase II clinical trial (NCT00942734), eligible patients were treated at a starting oral dose of everolimus 5 mg daily and erlotinib 150 mg daily which were tolerable doses previously defined [9]. A cycle was defined as 4 weeks. Treatment was continued until disease progression (PD), intolerable toxicity, investigator or patient decision. Patients were followed until PD and for 30 days following discontinuation of study treatment of toxicity monitoring.

Patients who did not complete at least 12 weeks of treatment without interruption of treatment of either everolimus or erlotinib for longer than 2 weeks, excluding patients with PD and patients without documented radiographic evaluations of their disease at 4 and 12 weeks were not considered fully evaluable and were replaced.

All patients provided written informed consent. The study protocol and all amendments were approved by the ethics body of MD Anderson Cancer Center. The study was conducted in accordance with good clinical practice guidelines, the Declaration of Helsinki, and all applicable local and federal regulations.

assessments

Tumor response was assessed per RECIST version 1.0 [11] by computed tomography (CT) or magnetic resonance imaging at 4 weeks, at 12 weeks after treatment initiation and then every 8 weeks until PD by the local investigator and an independent central radiology review (data not shown). Initial assessment of response required confirmation 28 days later. Safety was assessed according to the Common Terminology Criteria for Adverse Events, version 3.0, throughout the study by monitoring and recording all adverse events (AEs).

plasma cytokines and angiogenic factors

Of the 33 analyzed CAFs, 32 were analyzed at baseline, at 4 and 12 weeks from treatment initiation with commercially available multiplexed bead suspension arrays (EMD-Millipore, Billerica, MA). Carbonic anhydrase 9 (CA-9) was analyzed by ELISA (R&D Systems, Minneapolis, MN) [12].

mutation analysis

Mutation analysis was conducted for EGFR, KRAS, PI3KCA, BRAF, PTEN, CTNNB1, AKTI, P53, c-KIT, NRAS, MET, and GNAQ genes on paraffin-embedded cell blocks retrospectively obtained from the patients’ tumor at initial diagnosis by Sequenom analysis [13].

human papillomavirus testing

p16 immunohistochemistry (IHC) (p16 ink4a mouse anti-human clone E6H4), 1/3 of predilute (CINtec, Roche MTM Laboratories AG, Mannheim, Germany) was as previously reported [14]. Tumor cells were evaluated for p16 cytoplasmic and nuclear staining with diffuse, strong positivity >two thirds of the tumor cells reported as positive overexpression.

neutrophil gelatinase-associated lipocalin IHC

Neutrophil gelatinase-associated lipocalin (NGAL) IHC was carried out as described in supplementary material, available at Annals of Oncology online.

statistical analysis

Primary end points were objective response rate (ORR) by RECIST criteria [11] at 12 weeks or 12-week PFS. Secondary end points included toxicity, overall survival (OS) and CAF analysis. Based on historical 12-week PFS rate of ~40% [1] in the setting of platinum refractory HNSCC, we targeted a 12-week PFS rate of 45% or an ORR of 20%. No further exploration of this regimen would occur if the 12-week PFS rate was ≤45% and ORR ≤1%. One interim futility monitoring was carried out after the first 15 patients had been evaluated using a stopping rule based on Bayesian posterior probability for PFS and ORR. Formally if Pr (12-week PFS ≤0.45 | Data) ≤0.95 and 0/15 patients had objective response, then the trial would be stopped for futility (<4 of 15 patients progression-free at 12 weeks and no patients with objective response). Patients who did not reach the 12-week time point and discontinued without PD were replaced, while if they died they were counted as PD. If the true 12-week PFS rates were 20%, 30% or 45%, the respective probabilities of early termination of the trial were 65%, 30% and 4%. With a sample size of 35 and one interim analysis after 15 patients, the study had 90% power with a type I error rate of 0.031 to test the null (20%) versus alternative (45%) 12-week PFS rate. Associations of clinical outcomes with biomarkers were analyzed by transformation of logarithm to the base 2, and landmark analysis was carried out for change of the markers from baseline. The change over time of a CAF at each time point compared with baseline was calculated as the difference of CAF concentration at baseline to concentration at each time point on the log-transformed scale. Since these analyses were hypothesis generating and exploratory in nature, we did not adjust for multiple comparisons.
results

patients

Between September 2009 and October 2012, 36 patients were enrolled into the clinical trial, 35 were evaluable and one withdrew due to toxicity. Patients’ characteristics are shown in Table 1.

efficacy

Three of 35 (8%) assessable patients achieved partial response (PR) at 4 weeks, one of which was confirmed at 12 weeks. ORR rate [complete response (CR) + PR] at 12 weeks was 2.8%. Twenty-seven (77%) patients achieved disease stabilization (SD) at 4 weeks, 11 (31%) of which were confirmed at 12 weeks. The waterfall plot is shown in supplementary Figure S1, available at Annals of Oncology online. Median time to best response (PR + SD) was 8.14 weeks. Median duration of response (DOR) from first response to PD or death was 1.9 months. Twelve-week PFS was 49% and did not correlate with any of the patients characteristics (supplementary Table S1, available at Annals of Oncology online). Median PFS was 11.9 weeks (Figure 1A).

safety and tolerability

The most frequent grade ≥3 AEs were mucositis, diarrhea, skin rash, infections, head and neck edema (supplementary Table S2, available at Annals of Oncology online).

biomarker analysis

blood biomarkers. We measured 33 CAFs at each of the three time points (baseline, 4 and 12 weeks) (supplementary Table S3, available at Annals of Oncology online). Baseline high NGAL [hazard ratio (HR) = 4.57; 95% confidence interval (CI) 1.38–15.16; P = 0.01] and vascular endothelial growth factor (VEGF) (HR = 3.50; 95% CI 1.07–11.47; P = 0.01) levels were significantly associated with worse OS (Figure 1C and D).

High NGAL levels at 4 weeks were associated with worse PFS (HR = 11.11; 95% CI 2.13–57.9; P = 0.004).

High NGAL (HR = 7.52; 95% CI 1.45–38.92; P = 0.01), VEGF (HR = 1.43; 95% CI 1.05–1.95; P = 0.02), interleukin-6 (IL-6) (HR = 3.85; 95% CI 0.97–15.23; P = 0.05), CA-9 (HR = 1.18; 95% CI 1.03–1.36; P = 0.01) levels at 4 weeks were significantly associated with worse OS.

An increase of CA-9, interferon-γ (IFN-γ), granulocyte–macrophage colony-stimulating factor (GM-CSF), CD40 ligand (CD40L) and IL-17 levels from baseline to 4 weeks was significantly associated with worse PFS.

An increase of IL-1α, IL-1β, monocyte chemotactrant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) levels from baseline to 4 weeks was significantly associated with worse OS.

tumor biomarkers. A list of gene mutations for each patient included in the clinical trial is reported in supplementary Figure S1 and Table S4, available at Annals of Oncology online. Notably, a PI3KCA exon 20 mutation (codon 1047 CAT to CGT) and an EGFR exon 19 deletion were found in two separate cases with initial SD, which was not confirmed at 12 weeks. NGAL IHC expression was retrospectively carried out on 24 available FFPE tumor specimens at initial diagnosis. Protein expression was localized to the cytoplasm and median score was 55 (0–200). In 4 of 10 (40%) patients with high NGAL plasma levels by CAF analysis, a correspondent high NGAL protein expression was detected in the tumor specimen at diagnosis. No association between NGAL protein expression and ORR, DCR at 12 weeks, PFS or OS was observed.

HPV status was analyzed by p16 IHC. Nine (25%) of the 29 available tumoral tissues were p16 positive, 20 (56%) were negative. Eight of nine p16-positive tumors were from oropharyngeal primary and therefore were considered HPV positive. Median PFS and OS in HPV-positive patients were, respectively, 2.7 and 17.2 months and in HPV-negative patients, respectively, 2.9 and 7.6 months. No significant difference in PFS (HR = 1.3; 95% CI 0.5–3.0) was found for the HPV-positive patients when compared with the HPV-negative group; however, a numerical and clinical difference in OS was noted (HR = 0.3; 95% CI 0.07–1.3).

discussion

In this open-label, single-institution, phase II clinical trial of everolimus plus erlotinib in patients with metastatic HNSCC after standard platinum-based chemotherapy, the primary end points were not met, despite one confirmed PR at 12 weeks that met the prespecified threshold for expanding the study to full sample size.

The rationale for this combination was based on the frequent activation of the mTOR pathway in HNSCC [15], preclinical efficacy of mTOR inhibitors in both HPV-associated and HPV-negative settings [7], and enhanced biochemical effects and synergistic growth inhibition in vitro when mTOR and EGFR inhibitors were used in combination [16].

In our trial, ORR was 2.8%, 12-week DCR 34%, median DOR 1.9 months, PFS 8.14 weeks. In a previously published phase II trial of single-agent erlotinib in metastatic recurrent HNSCC ORR was 4.3%, SD was maintained in 38.3% patients for a median
duration of 16.1 weeks and PFS was 9.6 weeks [1]. Therefore, our results did not demonstrate an improvement in efficacy with everolimus addition to erlotinib treatment compared with historical control. Our data (supplementary Table S2, available at Annals of Oncology online) confirmed a tolerable toxicity profile as previously reported in the phase I/II clinical trial of the same drug combination in NSCLC [9]. Therefore, everolimus in combination with erlotinib has a more manageable toxicity profile when compared with the combination of other mTOR inhibitors, such as temsirolimus with erlotinib [17]. Both combinations include a 40%–50% lower dose of the mTOR inhibitor compared with maximum-tolerated dose.

HPV status did not statistically correlate with PFS and OS. Median PFS and OS for HPV-positive group were, respectively, 2.7 and 17.2 months, with a numerically and clinically meaningful difference for better OS compared with the HPV-negative group that was not statistically significant possibly due to small sample size.

Based on prior observations, we explored levels of CAFs associated with tumor invasion, inflammation, hypoxia, macrophage activation (supplementary Table S3, available at Annals of Oncology online) and found a strong association between high NGAL levels at baseline with worse OS (Figure 1D) and high levels of NGAL at 4 weeks with worse OS and PFS. NGAL IHC expression in diagnostic tumor samples collected retrospectively for 24 patients enrolled in the trial demonstrated mainly cytoplasmic localization. Only four patients with high NGAL plasma levels by CAF analysis showed a correspondent high NGAL tumor protein expression at diagnosis and no significant association between NGAL protein expression and ORR, 12-week DCR, PFS and OS was seen.

NGAL, also known as lipocalin 2, is a 25-kDa protein associated with matrix metalloproteinase-9 (MMP-9), belonging to the lipocalin superfamily, stored in specific granules of human neutrophils with a role in extracellular matrix remodeling and protection of MMP-9 from degradation [18]. In HNSCC, high NGAL expression is associated with increased invasiveness, metastasis and poor prognosis [19], while in NSCLC cells, acquired resistance to erlotinib is associated with strong up-regulation of the LCN2 gene encoding the NGAL protein; down-regulation of NGAL expression reversed resistance to erlotinib-induced apoptosis [20]. In our trial, although high plasma NGAL levels were associated with worse prognosis, the same was not true for tumor levels of the protein likely reflecting the use of archival and not immediately pretreatment specimens and possibly technical limitations such as tissue fixation.

Our data showed that markers of macrophage activation and inflammation (IL-6, MIP1α, MIP1β, CD40L, TNF-α) and of hypoxia (CA-9, VEGF) by CAF analysis were indicative of poor prognosis. In addition, an increase of CA-9, IFN-γ, GM-CSF,
CD40L and IL-17 levels from baseline to 4 weeks were significantly associated with worse PFS.

Similar prognostic role for markers of inflammation (IL-6, IL-8, VEGF, hepatocyte growth factor—HGF—and Gro-1) exists for patients with locoregionally advanced oropharyngeal HNSCC on combined modality chemotherapy and radiation therapy [21].

The interaction between hypoxia inducible factor-α/macrophage migration inhibitory factor and nuclear factor κ-light-chain enhancer of activated B/IL-6 axes plays an important role in the hypoxia-induced accumulation of CD11b+Gr-1+ myeloid cells and tumor growth in HNSCC [22–24]. In renal cell carcinoma and melanoma murine models, pharmacologic mTOR inhibition had both immune-stimulating and immune-suppressing effects [24] with a net effect resulting in decreased tumor growth. Temsirolimus enhanced the expression of CD8 lymphocyte markers associated with formation, both in vivo and in vitro. However, it also increased the proportion of T-regulatory cells expressing FoxP3 [25]. Therefore, we can hypothesize that everolimus may have deregulated chemokines that are crucial for T-cell differentiation, such as INF-γ, IL-1α, IL-β, IL-17, TNF-β, MCP-1, CD40L, GM-CSF, with subsequent increased T-regulatory cell number resulting into immunosuppression and tumor growth.

Wide next-generation sequencing profiling was not feasible for the brief responders and therefore, we were unable to potentially detect rare mutations affecting the TSC1 and TSC2, previously associated with responses to mTOR inhibitors [26].

In conclusion, the combination of erlotinib and everolimus showed little benefit in unselected patients with metastatic platinum-resistant HNSCC despite a manageable toxicity profile. Markers of tumor invasion and hypoxia identified a group of patients with particular poor prognosis.

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disclosure

The authors have declared no conflicts of interest.

references