Phase II Clinical and Exploratory Biomarker Study of Dacomitinib in Patients with Recurrent and/or Metastatic Squamous Cell Carcinoma of Head and Neck

Han Sang Kim,1,2 Hyeong Ju Kwon,3 Inkyung Jung,4 Mi Ran Yun,5 Myung-Ju Ahn,6 Byung Woog Kang,7 Jong-Mu Sun,6 Sung Bae Kim,8 Dok-Hyun Yoon,6 Keon Uk Park,9 Se-Hoon Lee,10 Yoon Woo Koh,11 Se Hun Kim,11 Eun Chang Choi,11 Dong Hoe Koo,12 Jin Hee Sohn,13 Bomi Kim,14 Nak-Jung Kwon,15 Hwan Jung Yun,16 Min Goo Lee,2 Ji Hyun Lee,17 Tae-Min Kim,18 Hye Ryun Kim,1 Joo Hang Kim,1 Soonmyung Paik,19,20 and Byoung Chul Cho1

Authors’ Affiliations: 1Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center; 2Department of Pharmacology, Pharmacogenomic Research Center for Membrane Transporters, Brain Korea 21 PLUS Project for Medical Science; 3Department of Pathology; 4Department of Biostatistics, Yonsei University College of Medicine, Seoul, Korea; 5JE UK Institute for Cancer Research, Gumi City, Kyungbuk, Korea; 6Samsung Medical Center, Seoul, Korea; 7Kyungpook National University Hospital, Daegu, Korea; 8Asan Medical Center, Seoul, Korea; 9Keimyung University, Daegu, Korea; 10Seoul National University Hospital, Seoul, Korea; 11Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea; 12Division of Hematology/Oncology, 13Department of Pathology, Kangbuk Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; 14Brain Korea 21 PLUS Project for Medical Science, Yonsei University; 15Macrogen Inc., Seoul, Korea; 16Division of Hematology-Oncology, Chungnam National University, Daejeon, Korea; 17Department of Oral Biology, College of Dentistry, Yonsei University, Seoul, Korea; 18Cancer Evolution Research Center, College of Medicine, The Catholic University of Korea, Seoul, Korea 19Division of Pathology NSABP, Pittsburgh, USA; 20Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul, Korea

Corresponding Author:
Byoung Chul Cho, M.D., Ph.D. Yonsei Cancer Center, Division of Medical Oncology, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea. Phone: 82-2-2228-8126; Fax: 82-2-393-3562; E-mail: cbc1971@yuhs.ac

Running title: Efficacy and exploratory biomarkers of dacomitinib in SCCHN.
Keywords: epidermal growth factor receptor, tyrosine kinase inhibitor, head and neck squamous cell carcinoma, biomarker

Count: Text 30 pages; 3 Tables; 3 Figures; Supplementary 5 Tables; Supplementary 3 Figures

Grant: This study was supported in part by a grant from the Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI12C1440, B. C. Cho) and the National Research Foundation of Korea (NRF) funded by the Korea government (MEST) (2012R1A2A2A01046927 to B. Cho).

Disclosure of Potential Conflicts of Interest
The authors have no potential conflicts of interest to disclose.
Translational Relevance

In this Phase II trial, treatment with dacomitinib, an irreversible tyrosine kinase inhibitor of EGFR, HER2 and HER4, showed objective response rate of 20.8%, progression-free survival of 3.9 months in patients with recurrent and/or metastatic squamous cell carcinoma of head and neck (R/M-SCCHN). Patients harboring phosphoinositide 3-kinase (PI3K)-pathway mutations or high inflammatory cytokine expression had shorter median progression-free survival and overall survival. Our data showed that dacomitinib has promising antitumor activity in heavily treated recurrent and/or metastatic SCCHN in patients without PI3K-pathway alteration or overexpression of inflammatory cytokines. Our findings might be important to select those patients who are most likely to benefit from dacomitinib in the design of future phase III clinical trials comparing the efficacy of dacomitinib with other palliative chemotherapy.
Abstract

Purpose

The goals of this study were to investigate the clinical activity, safety and biomarkers of dacomitinib, an irreversible tyrosine kinase inhibitor of EGFR, HER2 and HER4, in recurrent and/or metastatic squamous cell carcinoma of head and neck (R/M-SCCHN).

Experimental Design

Patients were eligible if the diseases were not amenable to curative treatment and had progressed on platinum-based chemotherapy, and were treated with dacomitinib 45mg/day. The primary endpoint was objective response rate by RECISTv1.1. Exploratory analysis included the characterization of somatic mutation, gene copy number, gene expression, p16\textsuperscript{INK4A} expression by immunohistochemistry, and investigation of their relationship with clinical outcomes.

Results

Forty-eight patients were evaluable for efficacy and toxicity. Ten patients (20.8%) had partial responses and 31 patients (65%) had stable diseases. The median progression-free survival (PFS) and overall survival (OS) were 3.9 months (95% CI, 2.9-5.0) and 6.6 months (95% CI, 5.4-10.3). Adverse events were mostly grade 1-2. Mutations in PI3K-pathway (\textit{PIK3CA}, \textit{PTEN}) and high expression of inflammatory cytokines (\textit{IL6}, \textit{IL8}, \textit{IL1A}, \textit{IL1B}, \textit{IL4}, and \textit{TNF}) were significantly associated with shorter PFS (2.9 v 4.9 months without mutations, \textit{P}=0.013; 2.8 v 9.9 months with low expression, \textit{P}=0.004). Those harboring PI3K-pathway mutations or high
inflammatory cytokine expression had shorter median OS (6.1 vs 12.5 months lacking PI3K-pathway mutations and with low inflammatory cytokine expression, $P=0.005$).

**Conclusions**

Dacomitinib demonstrated clinical efficacy with manageable toxicity in platinum-failed R/M-SCCHN patients. Screening of PI3K-pathway mutation and inflammatory cytokine expression may help identify which R/M-SCCHN patients are likely to gain benefit from dacomitinib.
Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is the seventh most common cancer worldwide (1). Although locally advanced disease is potentially curable with combined-modality therapy, 50~60% of patients developed locoregional or distant recurrence in 2 years (2). The prognosis of recurrent and/or metastatic SCCHN (R/M-SCCHN) is very poor, with a median survival of 6-10 months (3, 4). Platinum-based combination chemotherapy achieved modest response rates of 21~36% and failed to improve overall survival (OS) compared with single agent chemotherapies (5).

The epidermal growth factor receptor (EGFR) is a proto-oncogene regulating cell proliferation, angiogenesis, and metastasis. EGFR is almost universally expressed in SCCHN (6), and its overexpression is correlated with poor prognosis (7, 8). In this context, biologic agents targeting EGFR have been extensively studied in SCCHN (9-11). The current strategies for EGFR-targeted therapies in the treatment of R/M-SCCHN include EGFR-specific monoclonal antibodies (MoABs) and small molecule EGFR tyrosine kinase inhibitors (EGFR-TKIs).

Cetuximab, a chimeric anti-EGFR MoAB, was approved for the treatment of R/M-SCCHN as a single agent or in combination with chemotherapy (9, 10). The addition of cetuximab to platinum-based combination chemotherapy significantly improved objective response rate (ORR), progression-free survival (PFS) and OS compared with chemotherapy alone (9). Cetuximab as a monotherapy showed modest activity with ORR of 13% in R/M-SCCHN that progressed on platinum therapy (10).

Gefitinib and erlotinib are EGFR-TKIs that selectively block EGFR signaling
through competitive reversible binding at the EGFR-TK domain. In R/M-SCCHN, gefitinib or erlotinib showed relatively low ORR of 1.4%-10.6% with median OS of 5.5-8.1 months (12, 13). In a phase III study, gefitinib did not improve OS compared with methotrexate in R/M-SCCHN (13).

Clearly, only a minority of R/M-SCCHN patients respond to EGFR inhibitors. To maximize the clinical potential of EGFR inhibitors, there is an urgent need to identify molecular predictors of the efficacy of EGFR inhibitors. In an earlier study, SCCHN patients with high EGFR gene copy number showed higher ORR to erlotinib therapy than patients with low EGFR gene copy number. However, high EGFR gene copy number or EGFR overexpression failed to predict greater benefit from cetuximab combined with chemotherapy in R/M-SCCHN (14, 15). Recently, p16 status, a surrogate marker for human papillomavirus, has been suggested as a molecular predictor for panitumumab in combination with platinum combination for R/M-SCCHN (11). Comprehensive molecular profiling including somatic mutations, DNA copy number and mRNA expression may provide further opportunities to characterize a subset of patients who benefit the most from EGFR inhibitors.

Dacomitinib (PF-00299804) is a second-generation irreversible TKI of members of EGFR family including EGFR, ErbB2 and ErbB4 (16). A phase I study determined the dose of 45mg/d for clinical use. Dacomitinib significantly abrogates growth of SCCHN cells that are resistant to cetuximab (17). The purpose of this study was to investigate the clinical activity, safety and predictive biomarkers of dacomitinib in heavily-treated R/M-SCCHN.
Materials and Methods

Study Design

This was a multicenter, phase II study of dacomitinib monotherapy in patients with R/M-SCCHN who had progressed on platinum-based chemotherapy or was considered platinum-intolerant (ClinicalTrials.gov Identifier: NCT01449201). The primary objective was to evaluate the objective response rate. Secondary objective was to evaluate the clinical benefit (CB, PFS ≥4 months on dacomitinib), PFS, OS and the safety profile of dacomitinib therapy. Exploratory objectives were to evaluate whether tumor somatic mutation, copy number change, or gene expression are correlated with clinical outcomes.

All patients provided informed consent. The study was conducted in accordance with the Helsinki Declaration.

Study Population

Patients with histologically confirmed R/M-SCCHN were enrolled. Patients were at least age of 18 years, had an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0 to 2, had at least one measurable disease, had documented progressive diseases after platinum-based systemic chemotherapy for R/M-SCCHN, and had a life expectancy of at least 3 months. Chemotherapy-naïve patients with borderline renal function for platinum administration or ECOG-PS2 were allowed to enter the study at the physician’s discretion.

Patients were excluded if patients had received ≥3 lines of palliative chemotherapy for R/M-SCCHN, had nasopharyngeal cancer or had symptomatic brain metastases. Previous treatments with EGFR MoABs were allowed.

Treatment
Patients received continuous treatment with oral dacomitinib 45mg once daily until disease progression, death, or unacceptable adverse events (AEs). Treatment cycles were 28 days long. Drug doses were withheld and/or reduced for intolerable grade 2 or grade 3/4 toxic effects. A maximum of two dose-level reductions were permitted (30mg then 15mg). Patients who could not resume treatment after three-week delay were discontinued from the study.

**Patient assessment**

Response evaluations were defined according to RECIST 1.1 guidelines (18). Radiographic imaging was conducted at week 4, and every 8 weeks thereafter until disease progression or when clinically indicated. If a patient was documented as having a complete response (CR) or a partial response (PR), a confirmatory evaluation was performed after 4 weeks.

Safety assessments included physical examinations, documentation of AEs, laboratory measurements on day 1 of each cycle. AEs were graded according to the Common Terminology Criteria for Adverse Events version 4.0 (19).

**Exploratory Biomarker Analyses**

Fresh or archival tumor tissues were collected at baseline for biomarker analysis, which included the characterization of somatic mutations using the Ion Torrent AmpliSeq Cancer Hotspot Panel v2 (CHPv2), a next generation sequencing assay identifying multiple somatic mutations (2,800 Catalogue Of Somatic Mutations In Cancer [COSMIC] mutations from 50 genes); gene copy number using Nanostring nCounter® Cancer Copy Number Variation Assay (86 genes); gene expression using Nanostring nCounter® Human Cancer Reference Kit (230 genes) (Supplementary Table 1). The Nanostring method allows direct measurement of actual levels of
RNAs with no need for target amplification. The sensitivity and specificity of the AmpliSeq CHPv2 and nCounter® system have been previously validated (20, 21). Genomic DNA and mRNA were extracted from paraffin-embedded tumor blocks or fresh frozen tissues using standard extraction procedures.

Methods for gene set analysis, immunohistochemistry, fluorescence in situ hybridization (FISH), and enzyme-linked immunosorbent assay (ELISA) are described in the Supplemental Information.

Statistical Rationale for Study Design and Statistical analysis

A Fleming’s one-stage design was used to test the null hypothesis ($P_0$) with 5% significance level that the ORR is ≤5% versus the alternative hypothesis ($P_1$) that the ORR is ≥15%. Forty-four response-evaluable patients were required to provide 80% power to reject $P_0$ when the true ORR is 15%. Allowing for 10% loss to follow-up rate, it is anticipated that the total sample size is 49.

PFS was defined as the time from the first day of chemotherapy until the first disease progression or death from any cause. OS was defined as the time from the first day of chemotherapy to death from any cause. PFS and OS were summarized using the Kaplan-Meier method.

For the predictive biomarker analysis, we define PFS ≥4 months on dacomitinib as CB, because most salvage therapies with either cytotoxics or EGFR inhibitors in R/M-SCCHN have shown PFS of approximately 2 months (22, 23). The association of biomarkers with clinical outcomes was analyzed using a two-tailed Fisher’s exact test and a log-rank test, respectively. $P$-value ≤0.05 was considered significant. The significance was adjusted for the multiple tests when appropriate. Bonferroni correction for survival analysis and false discovery rate (FDR) for gene
expression analysis was performed using R version 3.0.1. Detailed method for corrected $P$-value calculation is described in the Supplemental Information.

**Results**

**Patient characteristics**

From January 2012 to March 2013, a total of 49 patients were enrolled (Supplementary Figure 1). One patient with external auditory canal cancer was incorrectly enrolled and excluded for the further analysis. Patient demographics are listed in Table 1. Majority of patients were male and ECOG-PS 0 or 1. The median age was 60.5 years. Approximately half of the patients had both locoregional and distant diseases in at least three organ sites. Only five patients (10%) received dacomitinib as the first-line systemic therapy because of ECOG-PS2 (n=3) or borderline renal function (n=2). Almost two thirds (67%) of patients had received all treatment modalities prior to enrollment. Five patients (10%) had received cetuximab as either definitive setting or palliative setting.

**Efficacy and Treatment Delivery**

Forty-eight patients were evaluated for response (Table 2). Overall, 20.8% of patients (10 of 48) had PR, 65% (31 of 48) had SD, and 13% (6 of 48) had PD as a best response. The waterfall plots of maximum percentage changes from baseline for 45 patients with follow-up images available are shown in Figure 1. The median duration of treatment was 15.6 weeks (interquartile range, 11.2 to 24.8 weeks). Reasons for treatment discontinuation were disease progression (n=46, 96%), patient withdrawal (n=1, 2%) and unacceptable toxicity (n=1, 2%; pneumonia). Eleven patients (23%) subsequently received BKM120, a pan-phosphoinositide-3-kinase (PI3K) inhibitor after progression on dacomitinib.
With a median follow-up of 8.4 months, median PFS and OS were 3.9 months (95% confidence interval [CI], 2.9-5.0) and 6.6 months (95% CI, 5.4-10.3). ECOG-PS 2 was significantly associated with shorter OS (2.9 v 8.8 months with ECOG-PS 0 or 1, P<0.01) with a trend toward shorter PFS (2.9 v 3.9 months with ECOG-PS 0 or 1, P=0.06). Prior cetuximab treatment was also significantly associated with shorter PFS (2.8 v 3.9 months without cetuximab, P=0.04), but was not associated with OS (6.4 v 8.2 months without cetuximab, P=0.42). There was no significant difference in ORR, PFS, and OS according to other clinical factors such as age, primary site, smoking history, or the number of prior chemotherapy.

Safety

Forty-eight patients were assessed for treatment-related AEs (Table 3). AEs were mostly grade 1 to 2 and easily manageable. The most common AEs were paronychia (65%) and diarrhea (52%). Treatment-related grade 3 AEs occurred in 6 patients. At least one dose interruption and reduction due to treatment-related AEs occurred in 24 patients (50%) and 9 (19%).

Detection of somatic aberrations

Biomarker results were available in 33 (somatic mutation, Figure 2A), 20 (gene expression, Figure 2B), 31 (DNA copy number, Figure 2C) and 34 patients (serum IL6 concentration, Figure 2D). Analysis of target-capture sequencing data identified a total of 58 point mutations, insertion, or deletions, previously implicated in COSMIC database (Supplementary Table 2).(24) Median sequencing depth in target regions was 730X. The frequency of somatic mutations was illustrated in Figure 2A. TP53 was the most frequently altered gene (58%) followed by CDKN2A (15%) and
PIK3CA (15%). The PI3K-pathway (PIK3CA, PTEN, and AKT1) mutations were frequently observed (8/33, 24%) and mutations in this pathway seemed to be mutually exclusive, except in one patient.

Gene expression profiling revealed that genes involved in inflammatory responses, such as tumor necrosis factor (TNF), interleukin-1 (IL1), IL4, IL6, IL8, secretory type II phospholipase A2 (PLA2G2A) and prostaglandin-endoperoxide synthase-2 (PTGS2, also known as COX2), were differentially expressed between patients with CB (n=8) and non-CB (n=12) \((P<0.001, \text{FDR}=0.006; \text{Supplementary Table 3, 4})\). Hierarchical cluster analysis demonstrated that 20 tumors could be classified into two clusters, each with distinctive expression pattern of inflammatory genes (Figure 2B). All patients with non-CB on dacomitinib belonged to cluster 1 with overexpression of inflammatory genes. Gene set analysis revealed that gene sets of inflammatory cytokines/mediators were significantly associated with cluster 1 (Supplementary Table 4). Three (15%) among 20 patients had both PI3K-pathway mutations and overexpression of inflammatory cytokines.

Frequent DNA copy number alterations were observed in genes related to cell cycle (CDKN2A, CCND1, CDK4, CDK6), cell differentiation (DCUN1D1), receptor tyrosine kinase (FGFR1, EGFR, ERBB2), and proliferation (PIK3CA) (Figure 2C, Supplementary Table 5).

**Association of Biomarkers with clinical outcomes**

Kaplan-Meier curves of Median PFS (3.9 months) and OS (6.6 months) were in Figure 3A. The frequency of somatic mutations in PIK3CA (E545K, E542K), PTEN (D326N, A120T, D107N, splice variant exon 3 c.165-1G>A), or AKT1 (D44G) was
significantly higher in the non-CB patients (39% v 7% with CB patients; \( P=0.046 \); Bonferroni-adjusted \( P=0.231 \); Figure 2A). Patients with these somatic mutations were significantly associated with shorter PFS (2.9 v 4.9 months without PI3K-pathway mutations; \( P=0.013 \); Bonferroni-adjusted \( P=0.065 \)) (Figure 3B). OS was not different according to PI3K-pathway mutations (Figure 3B). Patients without \( PIK3CA/PTEN/AKT1 \) mutation had unusually long durations of response (5.3, 6.1, 6.4, 6.8, 14.4, and 19.9 months). There is no objective response (PR or CR) in patients with PI3K-pathway mutations.

Overexpression of inflammatory cytokines/mediators was significantly associated with shorter PFS (2.8 v 9.9 months with low inflammatory cytokine expression; \( P=0.004 \); Bonferroni-adjusted \( P=0.016 \)) and shorter OS (6.1 v 20.3 months with low inflammatory cytokine expression; \( P=0.014 \); Bonferroni-adjusted \( P=0.056 \)), respectively (Figure 3C). Patients with low inflammatory cytokine expression also tended to have higher ORR (40% v 13% with high expression; \( P=0.25 \)), with unusually long durations of response (5.2, 5.8, 9.9, 14.4 and 19.9 months). Overall, patients who harbor \( PIK3CA/PTEN/AKT1 \) mutation or inflammatory cytokine overexpression were significantly associated with shorter PFS (2.9 v 6.1 months; \( P=0.001 \); Bonferroni-adjusted \( P=0.02 \)) and shorter OS (6.1 v 12.5 months; \( P=0.005 \); Bonferroni-adjusted \( P=0.1 \)) (Figure 3D). p16 positivity was not associated with PFS, but had a trend toward better OS (Supplementary Figure 2).

Patients with gene amplifications of receptor tyrosine kinases tended to have shorter PFS (1.0, 1.2, 2.1, and 2.9 months) (Figure 2C). A Representative case of \( FGFR1 \) amplification was illustrated using FISH (Supplementary Figure 3).
Serum IL6 level was measured in 34 patients at baseline and 14 days after the start of dacomitinib (Figure 2D). There was no difference in baseline IL6 level between patients with CB and non-CB ($P=0.986$). At day 14, however, there was a reduction of IL6 concentration compared with baseline only in patients with CB ($P=0.055$). Furthermore, serum IL6 concentration was significantly lower in patients with CB, compared with patients with non-CB at day 14 ($P=0.048$).
Discussion

In our study, characterization of somatic mutation, DNA copy number and gene expression was performed by high-throughput sequencing and digital count technology. Overall, this comprehensive biomarker analysis identified that the screening for phosphoinositide 3-kinase (PI3K)-pathway alteration and/or inflammatory gene expression could help identify subgroups most likely benefit from dacomitinib in R/M-SCCHN. Although our findings need to be validated in future randomized trials, our study demonstrated the applicability of a multi-omics approach for identification of novel biomarkers in the clinical trial setting of refractory R/M-SCCHN.

Compared with previous studies with single-agent EGFR inhibitors (10, 12, 13), patients enrolled in our study had noticeably more clinical factors associated with poor prognosis; 38% of patients had progressive disease after 2 prior chemotherapy regimens, 67% of patients had previously received all standard treatment modalities, and 50% of patients had at least three involved disease sites. In this population with poor prognosis, dacomitinib demonstrated encouraging clinical activity (confirmed ORR, 20.8%; PFS, 3.9 months). Our data compares favorably with the limited activity of cetuximab as a single agent in R/M-SCCHN (ORR 13%; time to progression of 70 days) (10).

Our data suggest that assessment of PI3K-pathway mutation may be used to guide therapy for R/M-SCCHN. The PI3K-pathway has been reported to be the most frequently mutated oncogenic pathway in SCCHN (25). In our study, the frequency of PI3K-pathway mutations was 24% (8 of 33 evaluable patients), which was comparable to those from the previous reports (25-28). Most of the PI3K-pathway
mutations were mutually exclusive, supporting an oncogenic role of PI3K-pathway in SCCHN. Oncogenic activation of PI3K-pathway contributes to resistance to EGFR inhibition in SCCHN preclinical models (29). Patients with PI3K-pathway mutations had a significantly shorter PFS, compared with patients without these mutations. A similar finding has recently been reported in breast cancer patients treated with lapatinib combined with letrozole (30). Given the controversial prognostic impact of PI3K-pathway mutation in various malignancies (31-33), a significantly longer PFS and durable objective response in our patients without PI3K-pathway mutations is most likely due to the favorable therapeutic effect of dacomitinib. Upon progression, 11 patients (23%) were enrolled into a phase II trial of BKM120 and one patient who had a PFS of 2.3 months to dacomitinib showed a PR to BKM120. Expectedly, a subsequent therapy with potential efficacy in patients with PI3K-pathway mutations might influence OS in our study.

Inflammation in the tumor microenvironment plays a critical role in tumor cell survival, angiogenesis, and resistance to anti-cancer therapy (34). In our study, overexpression of inflammatory genes contributed to resistance to dacomitinib. The overexpression of inflammatory cytokines/mediators can alter responses to dacomitinib via multiple mechanisms. These include induction of epithelial to mesenchymal transition (EMT) (35), suppression of anti-tumor immunity (36, 37), reprogramming of pro-tumoral microenvironment (36, 38), and upregulation of growth and survival pathway (39-43). IL1β modulates Snail and thereby downregulates E-cadherin expression in SCCHN, suggesting the role of inflammation on the induction of EMT (35). IL4 plays a key role in humoral and adaptive immunity and mediates immune evasion of cancer cells in tumor
microenvironment (36). IL8 signaling was shown to transactivate EGFR, promoting proliferation and survival of cancer cells through PI3K/mitogen-activated protein kinase (MAPK) pathway (39). In addition to its role in angiogenesis, IL8 induces a chemotactic infiltration of neutrophils into the tumor site, thereby generating protumoral microenvironment (38). Secretion of inflammatory cytokines has been known to constitutively activate nuclear factor-B (NF-κB) and signal transducers and activators of transcription 3 (STAT3), which directly regulate numerous genes, such as Bcl-2, cyclin D1 and c-myc, which have been associated with cell survival and chemoresistance (37, 40, 42, 44). TNF was reported to mediate constitutive activation of NF-κB in SCCHN cells (40). IL6 is a key downstream target of NF-κB and is a potent activator of STAT3 as well (45). Inhibition of PLA2G2A, a rate-limiting enzyme in tumor-stimulating eicosanoids production, reduced cell proliferation and NF-κB activity in non-small cell lung cancer (41). In addition, PLA2G2A induced proliferation of astrocytoma via activation of EGFR, suggesting its potential role in resistance to EGFR inhibitors (46).

IL6 has been known to induce resistance to anti-cancer therapy in different types of cancer (43, 45). To complement IL6 overexpression (>6-fold) in local tumor microenvironment, we compared the concentrations of plasma IL6 in patients with CB and non-CB on dacomitinib. Despite similar IL6 concentrations at baseline between two groups, its concentration at day 14 of dacomitinib was significantly higher in non-CB patients than in CB patients. This may suggest that IL6 induced by dacomitinib from tumor or stromal cells promoted resistance to dacomitinib in non-CB patients. This is in accordance with our previous report that dacomitinib induced STAT3 activation via autocrine IL6 production, promoting de novo resistance to
dacomitinib (43). It will be important to determine usefulness of plasma IL6 detection for monitoring therapeutic response to EGFR inhibitors in the future study.

Combination strategies targeting PI3K-pathway or inflammatory pathway can be a potential therapeutic option to overcome resistance and enhance efficacy of dacomitinib in R/M-SCCHN. Several agents targeting the PI3K-pathway (e.g. BYL719, XL147) (47, 48), inflammatory cytokines/mediators (e.g. ruxolitinib) (49) or COX-2 pathway (e.g. celecoxib) (50) in combination with EGFR inhibitors are under active investigation.

In conclusion, dacomitinib demonstrated promising efficacy with manageable toxicity in platinum-failed R/M-SCCHN patients. Screening of PI3K-pathway mutation and expression of inflammatory mediators may help identify R/M-SCCHN patients most likely benefit from dacomitinib.
Acknowledgement

This study was presented at the Biomarker Driven Clinical Trials session in American Association for Cancer Research Annual Meeting 2014, April 7th. This study was supported in part by a grant from the Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI12C1440, B. C. Cho) and the National Research Foundation of Korea (NRF) funded by the Korea government (MEST) (2012R1A2A2A01046927 to B. Cho). Pfizer provided dacomitinib. Pfizer did not provide funding for biomarker analysis.

We are very thankful to all members in Head & Neck Cancer Subcommittee of Korea Cancer Study Group (KCSG), JE UK Institute for Cancer Research, Macrogen Inc., and PhilKorea Inc. (Dr. Seong-Bin Kim) for supporting our work.
References


12. Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. Journal of clinical...
37. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role fo
### Table 1. Baseline patient characteristics (N=48)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>85</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>60.5 (30-82)</td>
<td></td>
</tr>
<tr>
<td><strong>Performance status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Smoker, pack-years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 10</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>32</td>
<td>67</td>
</tr>
<tr>
<td><strong>Time from initial diagnosis to study entry (month)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>19 (1-106)</td>
<td></td>
</tr>
<tr>
<td><strong>Primary site</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral cavity</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Larynx</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Maxillary sinus</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Disease status at study entry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locoregional</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>Distant</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Both</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td><strong>Number of involved disease sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>35</td>
</tr>
<tr>
<td>≥3</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td><strong>No. of prior chemotherapy regimens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>52</td>
</tr>
<tr>
<td>≥2</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td><strong>Previous chemotherapy regimen</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platinum-based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin alone</td>
<td>20</td>
<td>42</td>
</tr>
<tr>
<td>PF regimen</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>DP regimen</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>TPF regimen</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Chemotherapy alone</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Surgery + RT</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Surgery + CT</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Radiation + CT</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Surgery + RT + CT</td>
<td>32</td>
<td>67</td>
</tr>
</tbody>
</table>

Abbreviations: PF, cisplatin and 5-fluorouracil; DP, docetaxel and cisplatin; TPF, docetaxel, cisplatin and fluorouracil; RT, radiotherapy; CT, chemotherapy

*Chemotherapy was given as adjuvant, part of multimodality treatment, or palliative treatment
Table 2. Best response by treatment (N=48)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Partial response (confirmed)</td>
<td>10</td>
<td>20.8</td>
</tr>
<tr>
<td>Stable disease</td>
<td>31</td>
<td>64.6</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>6</td>
<td>12.5</td>
</tr>
<tr>
<td>Nonevaluable*</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Best overall response rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>20.8% (10.5 to 35.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Imaging study could not be done in two patients because of rapid disease progression.
*Response was not evaluable in one pt because of withdrawal from the study.
Table 3. Treatment-Related Adverse Events (N=48)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>All Grades</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>%</td>
<td>No. of patients</td>
</tr>
<tr>
<td><strong>Hematologic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>4</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Nonhematologic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acneiform dermatitis</td>
<td>21</td>
<td>44</td>
<td>1</td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>14</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Paronychia</td>
<td>31</td>
<td>65</td>
<td>1</td>
</tr>
<tr>
<td>PPE</td>
<td>17</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>22</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>General weakness</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>17</td>
<td>35</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>12</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>25</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td>Mucositis</td>
<td>21</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>AST/ALT elevation</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine elevation</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pain</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Infection*</td>
<td>3</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: PPE, Palmar-Plantar Erythrodynesthesia;
*Includes 1 pneumonia.
Figure Legends

Figure 1. Waterfall plot of maximum percentage changes from baseline in sum of the largest diameter of target lesions (N=45). Of 48 patients, three patients were excluded in this analysis because of the lack of follow-up imaging.

Figure 2. Somatic mutation, gene copy number, and gene expression profiles between patients with non-clinical benefit (progression-free survival [PFS] <4 months) and patients with clinical benefit (PFS ≥4 months). (A) somatic mutation (N=33), (B) clustering analysis of gene expression (N=20), (C) gene copy number (N=31), (D) Serum interleukin-6 level measured by the enzyme-linked immunosorbent assay (ELISA) (N=34). Samples were represented in ascending order of PFS in (A) somatic mutation and (C) gene copy number. The first row indicated subgroup according to PFS. The second row indicated CDKN2A (p16INK4A) expression by immunohistochemistry. Gene symbol and rate of mutation or copy number changes was shown in the left panel.

Figure 3. Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) according to PIK3CA/PTEN/AKT1 mutation or inflammatory gene expression. (A) PFS and OS (N=48), (B) PFS and OS according to PIK3CA/PTEN/AKT1 mutation status (N=33) (C) PFS and OS by inflammatory gene expression (N=20) (D) PFS and OS according to PIK3CA/PTEN/AKT1 mutation or inflammatory gene expression (N=33).
Figure 1

Best Percentage Changes From Baseline in Sum of Target Lesions

- Progression
- Stable disease
- Partial response (confirmed)
Figure 2

A

Group
P16

TP53 58%
CDKN2A 15%
ATM 3%
PIK3CA 15%
PTEN 9%
AKT1 3%
EGFR 6%
HRAS 6%
KRAS 6%
BRAF 3%
FGFR2 3%
FGFR3 12%
CTNNB1 6%
APC 3%

Somatic mutation
PFS < 4 months
PFS ≥ 4 months
Positive
Negative
Unknown

B

Cluster Group P16

IL1B
IL1A
IL8
TNF
PLA2G2A
PTGS2
IL6
IL4

Cluster 1
Cluster 2
Low
Gene expression
High
Positive
Negative
Unknown

C

Group
P16

CDKN2A
CCND1
CDK4
CDK6
DCUN1D1
FGFR1
EGFR
ERBB2
PIK3CA

≥ 6 copies
3 ~ 6 copies
Heterozygous deletion
Homzygous deletion
PFS < 4 months
PFS ≥ 4 months
Positive
Negative
Unknown

D

Serum IL-6 (pg/ml)

PFS < 4 months
PFS ≥ 4 months

P=0.986
P=0.048

P=0.92
P=0.055
Figure 3

A

Progression-Free Survival (%)

Time (months)

Number at risk

48 13 2 1 0

B

Progression-Free Survival (%)

Time (months)

Number at risk

Wild type 25 9 2 1 0
Mutant 8 0 0 0

PIK3CA/PTEN/AKT1 mutation positive (n=8)
PIK3CA/PTEN/AKT1 mutation negative (n=25)
Log-rank P = 0.013

C

Progression-Free Survival (%)

Time (months)

Number at risk

Low 5 3 2 1 0
High 15 2 0 0 0

High inflammatory gene expression (n=15)
Low inflammatory gene expression (n=5)
Log-rank P = 0.004

D

Progression-Free Survival (%)

Time (months)

Number at risk

Wild or low 12 7 2 0
Mutant or high 20 10 3 1

PIK3CA/PTEN/AKT1 mutation (+) or High inflammatory gene expression (n=20)
PIK3CA/PTEN/AKT1 mutation (-) or Low inflammatory gene expression (n=13)
Log-rank P = 0.001

Downloaded from clincancerres.aacrjournals.org on April 27, 2017. © 2014 American Association for Cancer Research.
Clinical Cancer Research

Phase II Clinical and Exploratory Biomarker Study of Dacomitinib in Patients with Recurrent and/or Metastatic Squamous Cell Carcinoma of Head and Neck

Han Sang Kim, Hyeong Ju Kwon, Inkyung Jung, et al.

Clin Cancer Res  Published OnlineFirst November 25, 2014.

Updated version: Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-1756

Supplementary Material: Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2014/11/26/1078-0432.CCR-14-1756.DC1

Author Manuscript: Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts: Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions: To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions: To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.