Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

Cancer Therapy: Clinical

Clinical Cancer Research

Molecular Alterations and Buparlisib Efficacy in Patients with Squamous Cell Carcinoma of the Head and Neck: Biomarker Analysis from BERIL-1

Denis Soulières[1], Lisa Licitra[2], Ricard Mesía[3], Éva Remenár[4], Shau-Hsuan Li[5], Andrey Karpenko[6], Marie Chol[7], Ying A. Wang[8], Nadia Solovieff[8], Laurence Bourdeau[9], Dalila Sellami[10], and Sandrine Faivre[11]

Abstract

Purpose: The preplanned exploratory analysis of the BERIL-1 trial presented here aimed to identify biomarkers of response to the combination of buparlisib and paclitaxel.

Patients and Methods: BERIL-1 was a multicenter, randomized, double-blind, placebo-controlled phase II study. Patients with recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) progressing on/after one previous platinum-based chemotherapy regimen in the recurrent or metastatic setting were treated with either buparlisib plus paclitaxel or placebo plus paclitaxel. Archival tumor tissue and ctDNA samples were analyzed for molecular alterations and immune infiltration using next-generation sequencing or immunohistochemistry.

Results: Biomarker analyses were performed in randomized patients ($n = 158$) with available biomarker data. The most frequently (>5%) mutated genes were *TP53*, *FAT1*, *TET2*, *KMT2D*, *PIK3CA*, *NOTCH1*, *NFE2L2*, *NOTCH2*, *CCND1*, and *CDKN2A*. Patients with SCCHN tumors (from various primary sites) having HPV-negative status (HR = 0.51), *TP53* alterations (HR = 0.55) or low mutational load (HR = 0.57) derived overall survival (OS) benefit with the combination of buparlisib and paclitaxel. OS benefit with this combination was also increased in patients with presence of intratumoral TILs ≥10% (HR = 0.51), stromal TILs ≥15% (HR = 0.53), intratumoral CD8-positive cells ≥5% (HR = 0.45), stromal CD8-positive cells ≥10% (HR = 0.47), or CD8-positive cells in invasive margins >25% (HR = 0.37). A trend for improved progression-free survival with the combination of buparlisib and paclitaxel was also observed in these patients.

Conclusions: The BERIL-1 biomarker analyses showed that patients with *TP53* alterations, HPV-negative status, low mutational load, or high infiltration of TILs or CD8-positive cells derived survival benefit with the combination of buparlisib and paclitaxel. *Clin Cancer Res; 24(11); 2505–16. ©2018 AACR.*

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is a heterogeneous disease with a high mortality rate at advanced stages (1–3). Substantial efforts have been devoted to understanding the molecular mechanisms contributing to the incidence and progression of this disease as well as to identifying potential prognostic and predictive markers (1, 4–7).

The phosphatidylinositol 3-kinase (PI3K) signaling pathway has been identified as one of the most frequently altered pathways in SCCHN (1, 2, 7). Alterations leading to activation of the PI3K pathway include gain-of-function mutations and amplifications in *PIK3CA*, PTEN alterations (such as loss of heterozygosity, inactivating mutations, or loss of expression), as well as overexpression or activation of downstream or upstream signaling molecules (1, 2, 5, 8–10). While *PIK3CA* mutations (~15%–20%) and reduced PTEN activity (identified in 6% to 82% of patients) are the most frequently reported molecular alterations of the PI3K pathway in SCCHN, overexpression of the upstream EGFR (occurring in about 90% of patients) is also expected to lead to PI3K pathway activation (4–7, 11–14). Activation of the PI3K pathway is likely to affect the response of SCCHN to paclitaxel, as PI3K activation was shown to contribute to paclitaxel resistance in other tumor types (15–17). In addition, inhibition of the PI3K pathway led to an increased efficacy of paclitaxel in ovarian cancer and malignant glioma cell lines. These observations, together with the reported activity of the PI3K inhibitor buparlisib (BKM120) in SCCHN preclinical models further support the key role of PI3K pathway in SCCHN (18).

Several other molecular alterations are frequently reported in SCCHN, including *TP53* mutation (~40%–75%), or amplification (~10%–30%), *CDKN2A* (p14/ARF) inactivation (~75%),

[1]Centre Hospitalier de l'Université de Montréal, Montréal, Canada. [2]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, and University of Milan, Milan, Italy. [3]Institut Català d'Oncologia—L'Hospitalet, Universitat de Barcelona, IDIBELL, Barcelona, Spain. [4]Országos Onkológiai Intézet, Budapest, Hungary. [5]Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan. [6]Leningrad Regional Oncology Dispensary, Saint Petersburg, Russian Federation. [7]Novartis Pharma AG, Basel, Switzerland. [8]Novartis Institutes for BioMedical Research, Cambridge, Massachusetts. [9]Novartis Pharma SAS, Rueil-Malmaison, France. [10]Novartis Pharmaceuticals Corporation, East Hanover, New Jersey. [11]Hôpitaux Universitaires Paris Nord Val de Seine, Paris, France.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

1. Presented as a poster at the 52nd American Society of Clinical Oncology (ASCO) Annual Meeting; June 3–June 7, 2016, Chicago, Illinois.

2. Oral presentation at the 6th International Conference on Innovative Approaches in Head and Neck Oncology (ICHNO); March 16–18, 2017, Barcelona, Spain.

Corresponding Author: Sandrine Faivre, Beaujon University Hospital, 100 Boulevard du Général Leclerc, Clichy 92110, France. Phone: 33631438843; Fax: 33140875487; E-mail: prof.faivre@gmail.com

doi: 10.1158/1078-0432.CCR-17-2644

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

Translational Relevance

Squamous cell carcinoma of the head and neck (SCCHN) is a heterogeneous disease with a high mortality rate at advanced stages. Although significant advances have been made toward identifying biomarkers of response, data on the clinical utility of these biomarkers are limited. The exploratory analysis of the BERIL-1 trial presented here aimed to identify biomarkers of response to the combination of buparlisib and paclitaxel, including biomarkers relevant to immune response. To our knowledge, this is one of the largest genetic landscape analyses focusing on recurrent or metastatic SCCHN (112 samples). Based on analysis of archival tumor tissue and ctDNA samples, we report that patients with *TP53* alterations, HPV-negative status, low mutational load, or high infiltration of TILs or CD8-positive cells derive survival benefit with the combination of buparlisib and paclitaxel. The current analysis also demonstrates the feasibility of using ctDNA for the assessment of HPV status and mutational load.

and cyclin D1 overexpression (~80%; refs. 4–7). Specifically, *TP53* mutations are known to be associated with a poor prognosis, as patients with *TP53*-altered tumors have decreased postsurgical survival (19). Various publications have also reported an association between *TP53* mutational status and response to platinum-based therapy in SCCHN; however, the nature of this association remains unclear. While some authors reported that *TP53* mutations may predict improved SCCHN cell response to cisplatin, or that *TP53*-mutant ovarian tumors were sensitive to taxane-platinum–based chemotherapy, other publications showed that functional or high-risk *TP53* mutations were associated with resistance to cisplatin-based therapies (20, 21). These observations highlight the importance of understanding the p53 cellular networks and their relation to responses to therapy (22–24).

Infection with the human papilloma virus (HPV), occurring in up to approximately 44% of cases, has been shown to contribute to SCCHN etiology and to influence outcomes (1, 2, 5–7, 25, 26). HPV-negative SCCHN tumors differ markedly from HPV-positive SCCHN in their clinical, immunological, and molecular characteristics (1, 2, 5, 6, 25, 27, 28). HPV-negative SCCHN also has a worse prognosis compared with HPV-positive SCCHN (1, 2, 5, 6, 25, 27–30). In addition, a strong association was reported between *TP53* alterations and HPV-negative SCCHN (7, 26).

The importance of immune markers in predicting outcome in SCCHN has been highlighted in recent studies. Infiltration of immune cells, especially $CD8^+$ cells, was previously shown to be associated with an improved response to chemoradiotherapy (31, 32). In addition, data suggest that expression of the programmed cell death ligand-1 (PD-L1) correlates with an improved clinical outcome under treatment with programmed cell death receptor-1 (PD-1) inhibitors (33, 34). Mutational load, identified by the total number of mutations present in a tumor specimen, is a potential biomarker for response to immunotherapy, as highly mutated tumors are more likely to harbor neoantigens, which make them targets of activated immune cells (35–37). Recent evidence suggests that high mutational load correlates with response to immune treatment in a variety of cancers (35–37). These data highlight the potential of immune markers and mutational load as prognostic and predictive markers in SCCHN and warrant further exploration.

Despite significant advances toward identifying biomarkers of response in SSCHN, data on the clinical utility of these biomarkers are limited, necessitating an investigation into finding additional biomarkers, specifically clonal events leading to oncogenic addiction or which could act as targets (38). Based on this observation, exploratory biomarker analyses were planned in the phase II BERIL-1 clinical trial in order to evaluate the impact on clinical outcome of molecular alterations relevant to SCCHN (such as PI3K activation or HPV infection), and to identify other molecular features potentially associated with response, through a broader analysis in tumor or ctDNA.

The clinical results of the phase II BERIL-1 trial have been reported in detail previously (18). Briefly, the BERIL-1 trial demonstrated that buparlisib (BKM120), an oral pan-PI3K inhibitor, in combination with paclitaxel improved clinical outcome with a manageable safety profile in patients with recurrent/metastatic SCCHN progressing on prior platinum-based therapy (18). Median progression-free survival (PFS) with buparlisib plus paclitaxel versus placebo plus paclitaxel was 4.6 months versus 3.5 months [hazard ratio 0.65 (95% CI, 0.45–0.95); $P = 0.011$; ref. 18]. Median overall survival (OS) with buparlisib plus paclitaxel versus placebo plus paclitaxel was 10.4 months versus 6.5 months (hazard ratio 0.72 (95% CI, 0.49–1.04; $P = 0.041$; ref. 18]. The purpose of the present publication is to describe the results of the BERIL-1 biomarker analyses. As this trial was not designed to address biomarker-related hypotheses, the analyses reported here should be considered exploratory and hypothesis generating.

Materials and Methods

Trial, patients, and tumor samples

BERIL-1 (NCT01852292) was a multicenter, randomized, double-blind, placebo-controlled phase II study (18). Trial and patient population have been described in detail previously (18). Briefly, adult patients with histologically or cytologically confirmed recurrent or metastatic SCCHN progressing on/after one previous platinum-based chemotherapy regimen in the recurrent or metastatic setting were stratified by prior lines of treatment (1 vs. 2) and study site (North America vs. rest of the world), and were treated with either buparlisib plus paclitaxel or placebo plus paclitaxel. The primary endpoint was PFS per RECIST v1.1. Secondary endpoints included OS, overall response rate (ORR), and safety. Formalin-fixed, paraffin-embedded (FFPE) archival tumor samples and plasma samples were obtained at screening. Appropriate patient consent was obtained before conducting the biomarker analyses on archival tumor and plasma sample. This study was conducted in accordance with recognized ethical guidelines (e.g., Declaration of Helsinki) and was approved by an institutional review board.

Analysis of molecular markers

Extraction and processing of tumor DNA from FFPE archival tissue and ctDNA from plasma samples was performed using standard procedures as described previously (39, 40). Sample analyses were conducted by sponsor laboratories, except HPV testing in archival tumor, which was conducted by HistoGeneX. Archival tumor and ctDNA samples were analyzed by next-generation sequencing (NGS) using a 44-gene panel and a 542-

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

gene panel, respectively (additional details in supplementary materials). *PIK3CA* mutations in archival tumor tissue were analyzed using polymerase chain reaction (PCR) or NGS. PTEN expression in archival tumor samples was assessed by immunohistochemistry (IHC) using the Cell Signaling Technology antibody (#9559). PTEN loss was defined as less than 10% cells expressing PTEN at a low level and no cell expressing PTEN at a medium or high level. HPV status was assessed by IHC (using the $p16^{INK4a}$ CINtec histology kit according to the manufacturer's instructions) in archival tumor tissue and by NGS in ctDNA samples. PD-L1 expression (Clone 22C3 PharmDx antibody; Dako #SK006), intratumoral and stromal CD8 expression (C8/144B antibody) and presence of TILs were determined by IHC. CD8 expression was also analyzed by IHC in invasive margins, when present in submitted tumor tissues. Mutational load was calculated as the number of nonsynonymous mutations detected per patient based on NGS analysis of ctDNA samples.

Only samples with adequate quantity, quality, and appropriate consent were included in the analysis. This resulted in 9.5% to 62% of unknown/missing data, depending on the biomarker tested, for analyses conducted in archival tumor (Table 1). Data obtained from ctDNA (HPV, *TP53*, and mutational load) were unknown/missing for 29.1% of patients, mostly because of consent unavailability.

Statistical analysis

Potential markers of response or resistance to treatment were evaluated in the current analysis. Overall survival (OS), PFS, and overall response rate (ORR) were estimated as described previously (18). OS, PFS, and ORR in patients having differential biomarker status were compared between treatment arms. The distribution and number of patients included in the analyses evaluating a potential association between biomarker status and OS, PFS, or ORR are described in Table 1. Cox proportional hazards models were used to compute HR and associated 95% CI for OS and PFS in the subpopulations defined by the individual or combined biomarkers. The thresholds selected for mutation load, CD8 expression, and presence of TILs were data driven, in the absence of validated thresholds for these in SCCHN. The thresholds selection was driven by the need to ensure an optimal separation of HR on OS, as well as by the need to obtain an easily measurable cutoff (e.g., 10%, 25%) while maintaining a meaningful balance between the number of patients included in the resulting biomarker subgroups. The median expression of CD8 was not used as the threshold in order to provide a clear determinant that could be easily used clinically. The CI for ORR was computed using the Clopper–Pearson exact method. Spearman correlation test was used to assess the relationship between continuous versus continuous/ordinal variables. Fisher exact test was used to assess the relationship between binary versus binary/ordinary variables. Wilcoxon rank-sum test was used to assess the relationship between continuous versus binary variables. No adjustment for multiple comparisons was performed.

Role of the funding source

The biomarker study design and plan for analysis was established in collaboration with the steering committee of the BERIL-1 study. The sponsor provided study drugs and participated in regulatory and ethics approvals, safety monitoring, data collection, and statistical analyses of the trial. All authors had full access to data for interpretation and analysis, were involved in development and approval of the manuscript, and had the final responsibility for the decision to submit for publication.

Results

Baseline biomarker status

A total of 158 patients were randomly assigned to either the buparlisib arm ($n = 79$) or placebo arm ($n = 79$). Biomarker analyses were performed in randomized patients with available biomarker data (Fig. 1). The baseline biomarker status in the buparlisib and placebo arms based on analyses of archival tumor tissue and ctDNA is summarized in Table 1. For each marker, frequencies of molecular alterations were calculated based on patients with a valid biomarker result. PI3K pathway was activated in 13.8% versus 16.1% of patients in the buparlisib versus placebo arms. The proportion of HPV-negative patients in the buparlisib versus placebo arm was 75.7% versus 84.9% based on archival tumor analysis and 77.4% versus 81.4% based on ctDNA analysis. *TP53* was altered in 57.8% versus 66.7% and 34.0% versus 42.4% of patients in the buparlisib versus placebo arms, based on analysis of archival tumor and ctDNA, respectively. In archival tumor, a higher frequency of *TP53* alterations and a lower frequency of *PIK3CA* alterations were observed among HPV-negative patients compared with HPV-positive patients (*TP53*, 70% vs. 21%; *PIK3CA*, 11% vs. 36%). Mutational load was low (<13 variants) in 75.6% vs. 79.7% of patients in the buparlisib versus placebo arm, based on ctDNA analysis. In addition, the proportion of patients with high immune markers expression (based on defined thresholds for PD-L1, TILs, and CD8) was numerically higher in the buparlisib arm, as compared with the placebo arm (Table 1).

Molecular landscape of SCCHN

ctDNA analyses of baseline plasma samples from 112 patients identified 10 genes mutated in more than 5% of patients with SCCHN (Fig. 2). These frequently mutated genes were (in decreasing order of frequency of mutations) *TP53*, *FAT1*, *TET2*, *KMT2D*, *PIK3CA*, *NOTCH1*, *NFE2L2*, *NOTCH2*, *CCND1*, and *CDKN2A*. *TP53* and *PIK3CA* were altered in 38% and 9% of patients respectively, with many of the identified mutations previously described in the literature or in the COSMIC database (41). These genes with frequency >5% were used to evaluate differences in the mutational profile of HPV-negative and HPV-positive patients. This analysis revealed a higher frequency of *TP53* alterations and a lower frequency of *PIK3CA* alterations in HPV-negative patients compared with HPV-positive patients (*TP53*, 44% vs. 17%; *PIK3CA*, 6% vs. 22%; Supplementary Table S1). Alterations in the gene encoding the p16/cyclin-dependent kinase inhibitor 2A (*CDKN2A*) were exclusively detected in patients with HPV-negative status.

Relationship between molecular alterations and response to the combination of buparlisib and paclitaxel

The PI3K activation status was defined, per protocol, as the presence of a *PIK3CA* mutation and/or a loss of PTEN expression. Among the patients with an evaluable PI3K activation status, the number of patients with PI3K pathway activation

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

Table 1. Baseline biomarker status by treatment arm

Biomarker[a]	Buparlisib + paclitaxel, *N* = 79 *n* (%)	Placebo + paclitaxel, *N* = 79 *n* (%)	All patients, *N* = 158 *n* (%)
Based on archival tumor tissue analysis			
PIK3CA alteration			
Yes	7 (11.7)	9 (14.5)	16 (13.1)
No	53 (88.3)	53 (85.5)	106 (86.9)
Unknown/missing	19	17	36
PTEN loss of expression			
Yes	1 (1.3)	1 (1.3)	2 (1.3)
No	74 (98.7)	77 (98.7)	151 (98.7)
Unknown/missing	4	1	5
PI3K pathway activation status[b]			
Activated	8 (13.8)	10 (16.1)	18 (15.0)
Nonactivated	50 (86.2)	52 (83.9)	102 (85.0)
Unknown/missing	21	17	38
HPV status			
Positive	17 (24.3)	11 (15.1)	28 (19.6)
Negative	53 (75.7)	62 (84.9)	115 (80.4)
Unknown/missing	9	6	15
TP53 alteration			
TP53 altered	26 (57.8)	26 (66.7)	52 (61.9)
TP53 nonaltered	19 (42.2)	13 (33.3)	32 (38.1)
Unknown/missing	34	40	74
PD-L1 expression status			
<1%	9 (28.1)	15 (53.6)	24 (40.0)
≥1%	23 (71.9)	13 (46.4)	36 (60.0)
Unknown/missing	47	51	98
Intratumoral TILs			
<10%	41 (58.6)	52 (71.2)	93 (65.0)
≥10%	29 (41.4)	21 (28.8)	50 (35.0)
Unknown/missing	9	6	15
Stromal TILs			
<15%	21 (30.4)	31 (43.1)	52 (36.9)
≥15%	48 (69.6)	41 (56.9)	89 (63.1)
Unknown/missing	10	7	17
Intratumoral CD8 expression			
<5%	36 (58.1)	41 (66.1)	77 (62.1)
≥5%	26 (41.9)	21 (33.9)	47 (37.9)
Unknown/missing	17	17	34
Stromal CD8 expression			
<10%	19 (31.1)	26 (42.6)	45 (36.9)
≥10%	42 (68.9)	35 (57.4)	77 (63.1)
Unknown/missing	18	18	36
CD8 expression in invasive margins			
<25%	38 (69.1)	38 (82.6)	76 (75.2)
≥25%	17 (30.9)	8 (17.4)	25 (24.8)
Unknown/missing	24	33	57
Based on ctDNA analysis			
HPV status			
Positive	12 (22.6)	11 (18.6)	23 (20.5)
Negative	41 (77.4)	48 (81.4)	89 (79.5)
Unknown/missing	26	20	46
TP53 alteration			
TP53 altered	18 (34.0)	25 (42.4)	43 (38.4)
TP53 nonaltered	35 (66.0)	34 (57.6)	69 (61.6)
Unknown/missing	26	20	46
Mutational load			
Low mutational load (<13 variants	39 (75.6)	47 (79.7)	86 (76.8)
High mutational load (≥13 variants)	14 (26.4)	12 (20.3)	26 (23.2)
Unknown/missing	26	20	46

Abbreviations: CD8, cluster of differentiation 8; PD-L1, programmed death ligand 1; *PIK3CA*, phosphatidylinositol 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; and TILs, tumor-infiltrating lymphocytes.

[a]For each marker, frequencies of reported alterations were calculated based on patients with a valid biomarker data. The proportions of patients with unknown/missing status (excluded from the frequency calculation) are as follows: *PIK3CA* 22.8%; PTEN loss 3.2%; PI3K activation 24.1%; HPV in archival tumor 9.5%; *TP53* alteration in archival tumor 46.8%; PD-L1 62%; intratumoral TILS 9.5%; stromal TILS 10.8%; intratumoral CD8 21.5%; stromal CD8 22.8%; CD8 in invasive margins 36.1%; HPV, *TP53,* and mutational load in ctDNA 29.1%.

[b]PI3K activation status was derived from *PIK3CA* mutation status and PTEN expression status. The PI3K status was considered activated if a *PIK3CA* alteration was identified and/or if PTEN expression was lost.

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

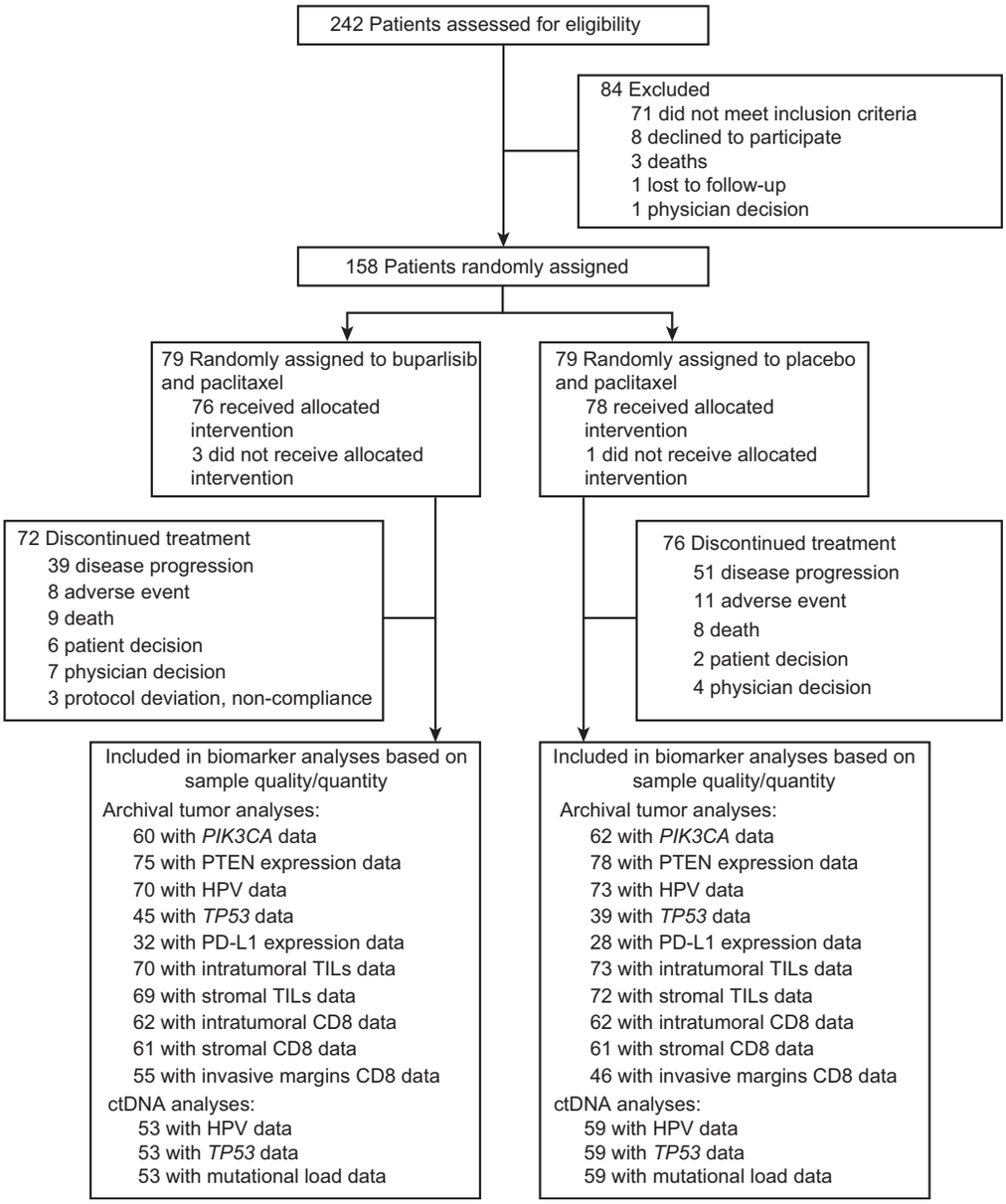

Figure 1.
CONSORT flow diagram.

was small (18 out of 120; Table 1), which weakened the statistical analyses performed to evaluate a possible relationship between the PI3K activation status and the clinical outcome. These analyses did not suggest a difference in OS between the buparlisib and placebo arms in the PI3K-activated subgroup (Fig. 3). A trend for improved PFS and ORR with buparlisib and paclitaxel was observed in the PI3K-activated subgroup (Table 2), but this trend was similar to the improved PFS and ORR observed in the PI3K-nonactivated subgroup and in the full population. As mentioned, these observations should be taken with caution, as the small number of patients with PI3K activation resulted in noninformative statistical results in this subgroup. It was not possible to perform an equivalent analysis in ctDNA because loss of PTEN expression cannot be obtained from ctDNA.

The sets of patients included in the analyses based on HPV or *TP53* status in archival tumor or ctDNA were driven by biomarker data availability. The HPV status was obtained from 143 patients on archival tumor and from 112 patients on ctDNA, with 105 patients having an HPV status obtained on both sample types. The *TP53* status was obtained from 84 patients on archival tumor and from 112 patients on ctDNA, with 63 patients having a *TP53* status obtained from both sample types. Based on patients with data available in the two sample types, the concordance between data obtained on archival or ctDNA was of 89% for HPV status and of 63% for *TP53* status.

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

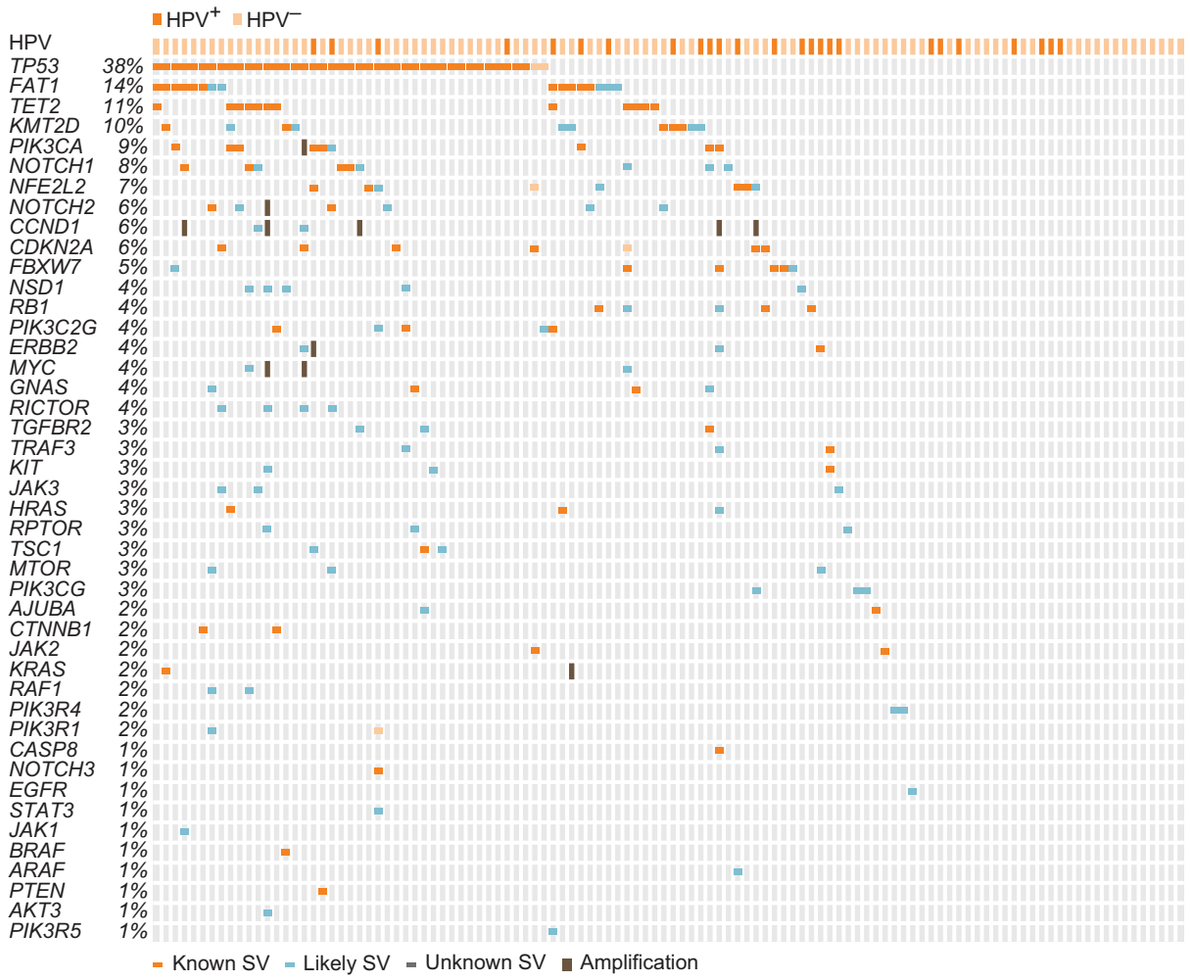

Figure 2.
Most frequent gene alterations in ctDNA at screening.

Patients with tumors having HPV-negative status, *TP53* alterations or low mutational load derived clinical benefit with the combination of buparlisib and paclitaxel. In patients with HPV-negative tumors, the median OS was longer in the buparlisib arm based on both archival tumor [HR = 0.61 (95% CI, 0.4–0.92)] and ctDNA analysis [HR = 0.51 (95% CI, 0.31–0.84)]. OS benefit with the combination of buparlisib and paclitaxel was also observed in patients with *TP53* alterations [archival tumor analysis HR = 0.52 (95% CI, 0.28–0.99) and ctDNA analysis HR = 0.55 (95% CI, 0.27–1.12)] and in patients with low mutational load [HR = 0.57 (95% CI, 0.34–0.97); Fig. 3].

Buparlisib combined with paclitaxel prolonged PFS in patients having tumors with HPV-negative status [HR = 0.58 (95% CI, 0.38–0.89)] or *TP53* alterations [HR = 0.45 (95% CI, 0.23–0.89)] based on archival tumor analysis. A similar non-significant trend of improved PFS with the combination of buparlisib and paclitaxel was observed in ctDNA analyses for HPV-negative patients [HR = 0.62 (95% CI, 0.37–1.02)] and for patients with a low mutational load [HR = 0.63 (95% CI, 0.37–1.08)] (Table 2).

ORR was numerically greater in patients in the buparlisib arm compared with the placebo arm irrespective of molecular status. However, the magnitude of benefit with the combination of buparlisib and paclitaxel tended to be greater in patients with HPV-negative status, based on archival tissue analysis (Table 2).

Relationship between immune infiltration and response to the combination of buparlisib and paclitaxel

A potential relationship between PD-L1 expression and treatment benefit was explored, using the 1% expression cutoff previously described in the literature (33). However, due to the small number of samples in the PD-L1 expression analysis ($n = 60$), no definite conclusion could be drawn regarding the association between PD-L1 expression and treatment benefit (Fig. 4; Table 2).

An improved OS benefit with the combination of buparlisib and paclitaxel was observed in patients showing immune cell infiltration, regardless of the compartment where this infiltration occurred. OS benefit with this combination was increased in patients with the presence of intratumoral TILs $\geq$10% [HR = 0.51 (95% CI, 0.26–1.00)], stromal TILs $\geq$15% [HR = 0.53, (95%

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

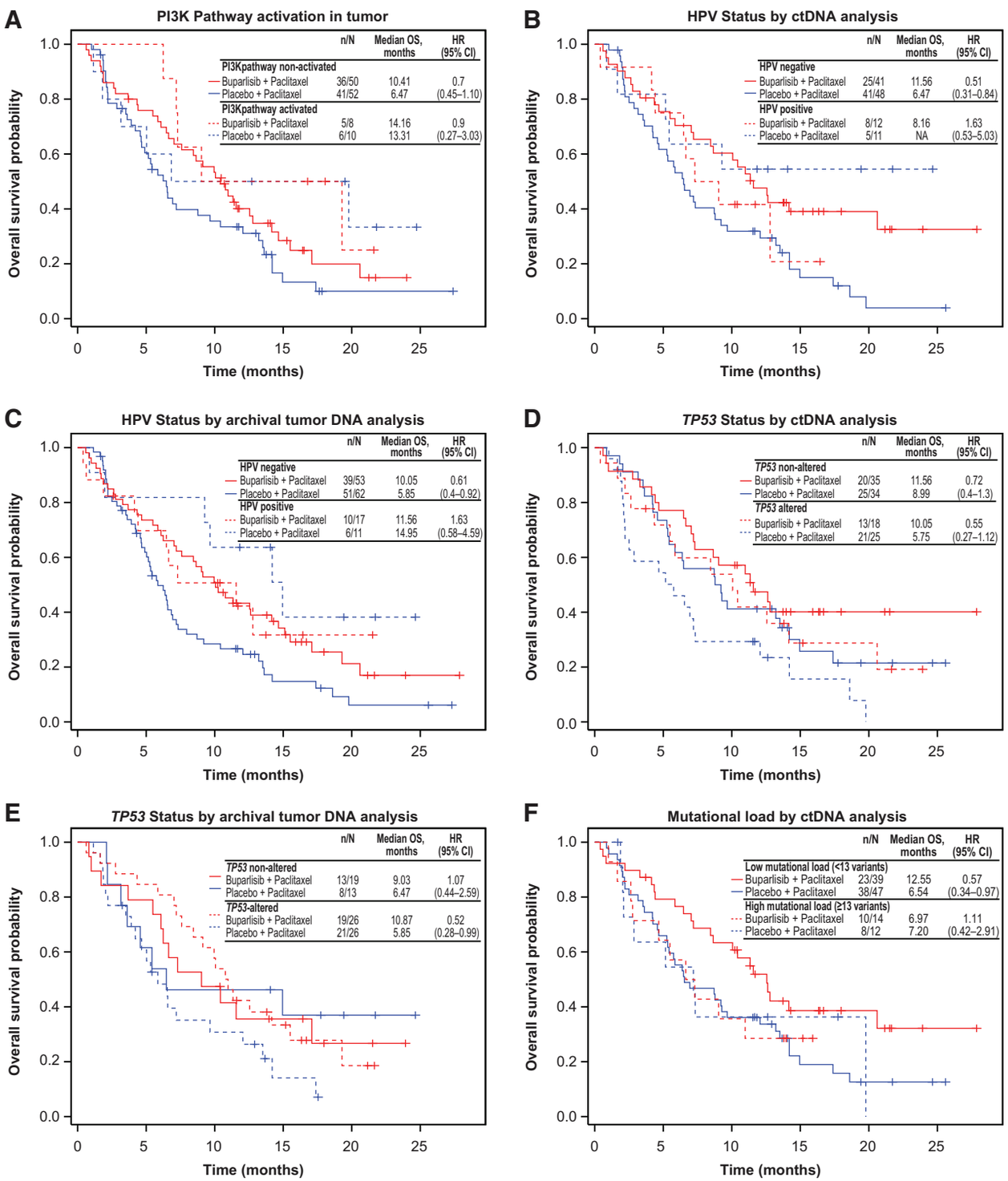

Figure 3.

Kaplan–Meier curves for overall survival by HPV, *TP53,* and mutational load status. **A,** PI3K pathway activation in tumor. **B,** HPV status by ctDNA analysis. **C,** HPV status by archival tumor DNA analysis. **D,** *TP53* status by ctDNA analysis. **E,** *TP53* status by archival tumor DNA analysis. **F,** Mutational load by ctDNA analysis.

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

Table 2. Progression-free survival and overall response rate by biomarker status based on archival tissue and ctDNA analyses

		PFS			ORR% (95% CI)	
Biomarker[a]	**Sample**	**Buparlisib + paclitaxel (PFS in months)**	**Placebo + paclitaxel (PFS in months)**	**HR (95% CI)**	**Buparlisib + paclitaxel**	**Placebo + paclitaxel**
HPV status	Archival tumor					
Positive		4.99	5.13	1.06 (0.40–2.79)	35.3 (14.2–61.7)	27.3 (6.0–61.0)
Negative		3.71	2.33	0.58 (0.38–0.89)	39.6 (26.5–54.0)	11.3 (4.7–21.9)
HPV status	ctDNA					
Positive		3.66	3.68	1.23 (0.44–3.41)	25.0 (5.5–57.2)	18.2 (2.3–51.8)
Negative		4.63	3.61	0.62 (0.37–1.02)	43.9 (28.5–60.3)	18.8 (8.9–32.6)
TP53 alteration	Archival tumor					
TP53 altered		5.03	2.17	0.45 (0.23–0.89)	38.5 (20.2–59.4)	7.7 (0.9–25.1)
TP53 nonaltered		3.78	3.61	0.74 (0.33–1.64)	42.1 (20.3–66.5)	23.1 (5.0–53.8)
TP53 alteration	ctDNA					
TP53 altered		3.48	2.17	0.76 (0.37–1.53)	33.3 (13.3–59.0)	8.0 (1.0–26.0)
TP53 nonaltered		5.03	3.71	0.81 (0.46–1.43)	42.9 (26.3–60.6)	26.5 (12.9–44.4)
Mutational load	ctDNA					
Low mutational load (<13 variants)		4.90	3.61	0.63 (0.37–1.08)	43.6 (27.8–60.4)	19.1 (9.1–33.3)
High mutational load (≥13 variants)		2.94	3.71	1.23 (0.51–2.97)	28.6 (8.4–58.1)	16.7 (2.1–48.4)
PI3K pathway activation status	Archival tumor					
Activated		5.32	3.73	0.67 (0.21–2.14)	50.0 (15.7–84.3)	10.0 (0.3–44.5)
Nonactivated		4.9	3.45	0.63 (0.4–0.99)	38.0 (24.7–52.8)	13.5 (5.6–25.8)
PD-L1 expression status	Archival tumor					
<1		3.68	3.94	0.64 (0.24–1.68)	77.8 (40.0–97.2)	13.3 (1.7–40.5)
≥1		4.90	3.45	0.77 (0.34–1.77)	39.1 (19.7–61.5)	15.4 (1.9–45.4)
Intratumoral TILs	Archival tumor					
<10%		3.71	3.52	0.81 (0.51–1.29)	39.0 (24.2–55.5)	15.4 (6.9–28.1)
≥10%		4.99	2.20	0.53 (0.26–1.07)	41.4 (23.5–61.1)	9.5 (1.2–30.4)
Stromal TILs	Archival tumor					
<15%		3.71	3.55	0.91 (0.47–1.74)	33.3 (14.6–57.0)	16.1 (5.5–33.7)
≥15%		4.90	2.20	0.51 (0.31–0.84)	43.8 (29.5–58.8)	9.8 (2.7–23.1)
Intratumoral CD8 expression	Archival tumor					
<5%		3.52	3.52	0.78 (0.46–1.31)	30.6 (16.3–48.1)	14.6 (5.6–29.2)
≥5%		5.32	2.20	0.42 (0.21–0.86)	50.0 (29.9–70.1)	14.3 (3.0–36.3)
Stromal CD8 expression	Archival tumor					
<10%		3.04	2.37	0.72 (0.35–1.46)	31.6 (12.6–56.6)	11.5 (2.4–30.2)
≥10%		5.32	2.37	0.46 (0.27–0.80)	42.9 (27.7–59.0)	14.3 (4.8–30.3)
CD8 expression in invasive margins	Archival tumor					
≤25%		3.61	2.33	0.63 (0.38–1.05)	39.5 (24.0–56.6)	7.9 (1.7–21.4)
>25%[a]		7.82	5.54	0.42 (0.14–1.24)	41.2 (18.4–67.1)	12.5 (0.3–52.7)

Abbreviations: CD8, cluster of differentiation 8; ctDNA, circulating tumor DNA; HPV, human papilloma virus; PD-L1, programmed death ligand 1; TILs, tumor infiltrating lymphocytes.

[a]Refer to Table 1 for the number of patients (*n*) included in each biomarker/treatment subgroup.

CI, 0.33–0.85)], intratumoral CD8-positive cells ≥5% [HR = 0.45 (95% CI, 0.23–0.86)], stromal CD8-positive cells ≥10% [HR = 0.47, (95% CI, 0.28–0.79)] or CD8-positive cells in invasive margins >25% [HR = 0.37 (95% CI, 0.14–0.94)] (Fig. 4). Buparlisib combined with paclitaxel also prolonged PFS in patients showing presence of intratumoral TILs ≥10% [HR = 0.53 (95% CI, 0.26–1.07)], stromal TILs ≥15% [HR = 0.51 (95% CI, 0.31–0.84)], intratumoral CD8-positive cells ≥5% [HR = 0.42 (95% CI, 0.21–0.86)] or stromal CD8-positive cells ≥10% [HR = 0.46 (95% CI, 0.27–0.80)]. Patients in the buparlisib arm had numerically higher ORR, irrespective of immune infiltration status (Table 2).

Relationship between molecular alterations and immune status

An analysis was conducted to explore the relationship between the different markers shown to be associated with improved outcome with the combination of buparlisib and paclitaxel (Supplementary Fig. S1). A clear association was observed between the presence of *TP53* alterations and HPV-negative status in both tumor and ctDNA analyses. Signs of a possible association between high mutational load and an HPV-negative status or the presence of *TP53* alteration were observed, which would require further exploration before drawing any conclusion.

A clear relationship was also observed across the different markers of immune infiltration. Associations were observed between the proportion of CD8-positive cells and the proportion of TILs across the different compartments. There was, however, no clear relationship between the molecular alterations and the markers of immune infiltration, with only anecdotal associations observed between these two groups.

Discussion

The current analysis explored the genetic landscape of SCCHN and the correlation of treatment efficacy with various biomarkers. To our knowledge, this is one of the largest genetic landscape analyses focusing on recurrent or metastatic SCCHN (112 samples). The genetic profile obtained from SCCHN samples in this analysis was consistent with previous publications (1, 7, 26, 42). Genes previously reported as frequently mutated in SCHNN, such as *TP53*, *FAT1*, *CDKN2A*, *PIK3CA*, or *NOTCH1*, were also among the most frequently altered genes in this study. In line with

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

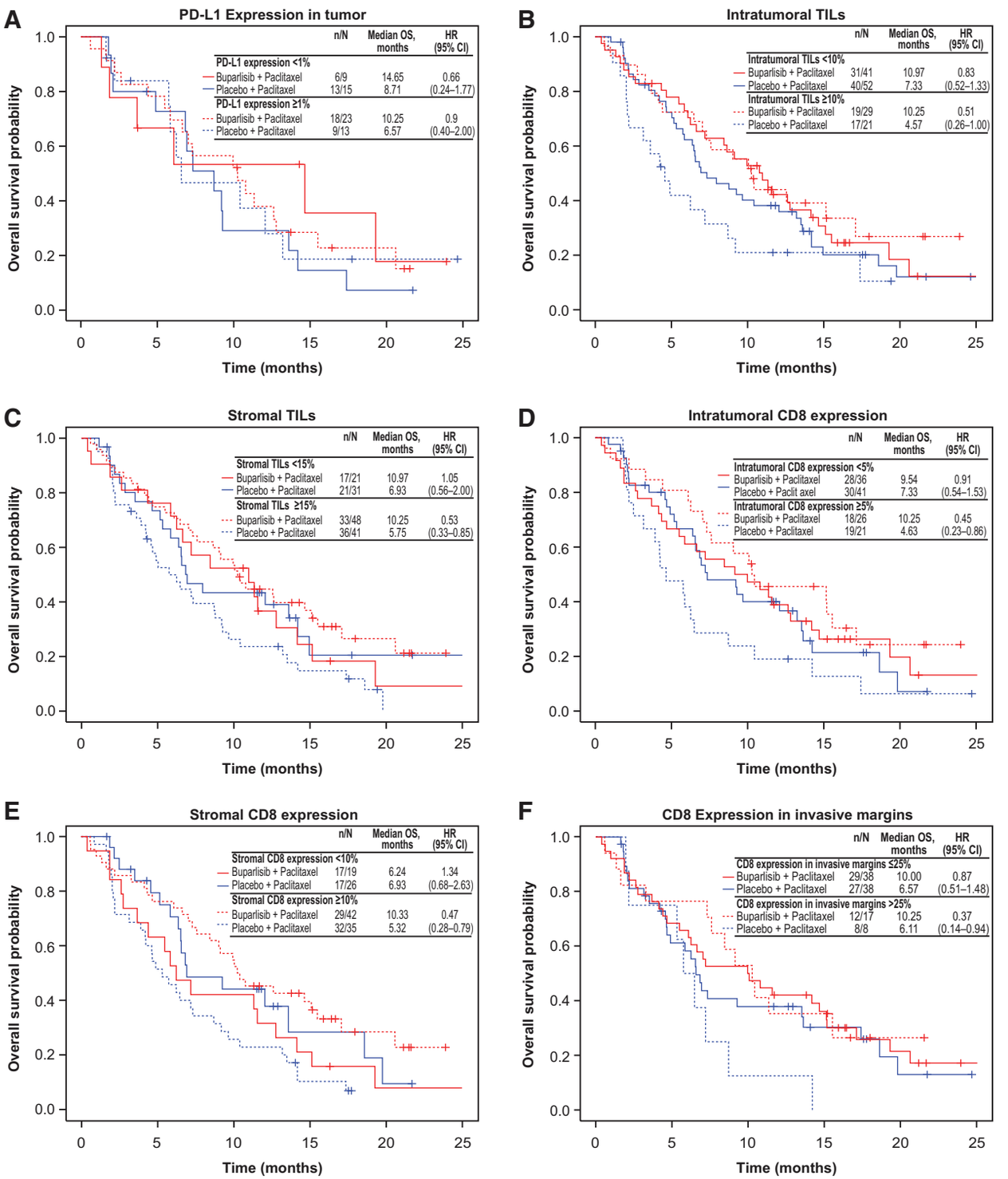

Figure 4.
Kaplan–Meier curves for overall survival by status of immune markers. **A,** PD-L1 expression in tumor. **B,** Intratumoral TILs. **C,** Stromal TILs. **D,** Intratumoral CD8 expression. **E,** Stromal CD8 expression. **F,** CD8 expression in invasive margins.

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

those previous reports, we also observed a higher frequency of *TP53* alterations and a lower frequency of *PIK3CA* alterations in HPV-negative patients compared with HPV-positive patients, and we identified *CDKN2A* alterations exclusively in HPV-negative patients.

The prognostic nature of *TP53* alterations and HPV status in SCCHN is well documented with poor outcomes reported for patients with *TP53*-altered tumors and patients with HPV-negative SCCHN (1, 2, 5, 6, 19, 25, 27, 28). In BERIL-1, HPV infection was also identified as a positive prognostic factor in the placebo arm (OS in patients with HPV-positive vs. HPV-negative status was 14.95 months vs. 5.85 months). However, patients with tumors having *TP53* alterations or HPV-negative status derived survival benefit with the combination of buparlisib and paclitaxel, suggesting that PI3K inhibition may improve outcome in this subset of patients historically characterized by a poor clinical outcome. The improved outcome in this population may be partly explained by the fact that alterations in p53 are expected to disrupt the ability of p53 to downregulate the PI3K pathway. As nonaltered p53 was shown to increase PTEN expression and to reduce PI3Kα expression, it is likely that alterations in p53 may contribute to PI3K activation, hence potentially increasing the sensitivity of the p53-altered cells to PI3K inhibitors (43).

While analyses on archival tumor and ctDNA provided similar trends for the associations of biomarkers with clinical outcome, some variations were observed in the magnitude and significance of these observations. This can be explained by (i) the fact that patient sets included in archival tumor and ctDNA analyses are different and (ii) the concordance rate between the biomarker status identified in archival tumor and ctDNA. In this study, we observed an 89% concordance between the HPV status obtained from archival tumor or ctDNA. This concordance rate would, however, need to be confirmed on an independent set of samples, because the cutoff for determining HPV status was chosen to optimize sensitivity and specificity vs. HPV status in archival tumor, which may lead to overestimating the current concordance rate. For *TP53*, a 63% concordance was observed between the status obtained from archival tumor and ctDNA. This concordance rate was mainly driven by the fact that among the patients with a *TP53* alteration identified in archival tumor, only 50% (18/36) also had a *TP53* alteration identified in ctDNA, likely due to a low tumor contribution to plasma. In addition, the *TP53* concordance rate was also affected by *TP53* mutations detected in ctDNA, but not in archival tumor, for five patients, potentially due to the occurrence or expansion of new clones between the initial archival tumor collection and the collection of ctDNA.

The current analysis also demonstrated the feasibility of using ctDNA for the assessment of HPV status and mutational load. While mutational load should ideally be estimated from whole exome sequencing, Chalmers and colleagues showed that mutational load can be reasonably estimated using smaller targeted NGS panels ($\geq$1.1 MB coding region; ref. 44). The NGS panel used in our study targets approximately 1.5 MB of coding region and was thus considered as an acceptable approach to estimate mutational load on ctDNA. In this BERIL-1 study, we observed a relationship between a low mutational load in ctDNA and an improved response to the combination of buparlisib and paclitaxel compared with the response to the combination of placebo and paclitaxel. Although recent evidence suggests that a high mutational load could be associated with better response to treatment with checkpoint inhibitors in a variety of cancers, this is, to our knowledge, the first study showing an association between mutational load and response to a PI3K inhibitor (35–37).

Emerging evidence suggests that the immune system plays a critical role in the development and evolution of SCCHN. In recent analyses, higher TIL levels, especially $CD8^+$ TILs, have been shown to be associated with improved outcomes (31, 45–47). Recent evidence also highlights the importance of a comprehensive evaluation of tumor cells and tumor-infiltrating immune cells when determining the prognostic relevance of immune markers such as PD-L1 (48, 49). In BERIL-1, patients with above-threshold levels of TILs and $CD8^+$ cells tended to derive superior survival benefit from the combination of buparlisib and paclitaxel, compared with the combination of placebo and paclitaxel. This relationship between response to treatment and immune infiltration status may be linked to the potential ability of PI3K inhibition to prime the immune system. PI3Kδ inhibition was previously suggested to promote immune-mediated elimination of cancer. In addition, inactivation of PI3Kγ in host mice was shown to be associated with an increased tumor infiltration of $CD4^+$ and $CD8^+$ T lymphocytes (50, 51). Buparlisib, which was recently suggested to alleviate tumor immune suppression by promoting IFNγ secreting, antitumor T cells, could potentially act on immune tumor response through inhibition of PI3Kγ and/or PI3Kδ (45).

Although the current analysis provides valuable insights into the potential biomarkers associated with response to the combination of buparlisib and paclitaxel in SCCHN, some limitations of the analysis need to be considered when interpreting this data. The key limitations of the current study include the small number of patients with alterations in the PI3K pathway, as well as the high proportion of unknown/missing in some biomarker subgroups. Additionally, the biomarker data were generated retrospectively in patient subgroups with valid biomarker data and the patients were thus not stratified based upon biomarkers. Furthermore, the thresholds selected for mutational load and some of the immune marker analyses were data driven and established after exploratory analyses of different thresholds. This could lead to overoptimized estimate of treatment effect in the biomarker subgroup. Validation of the association between the biomarker subgroups and efficacy in an independent cohort is warranted.

In conclusion, the BERIL-1 biomarker analyses showed that patients with *TP53* alterations, HPV-negative status, low mutational load or above-threshold infiltration of TILs or CD8-positive cells derived survival benefit with the combination of buparlisib and paclitaxel. Further investigations are warranted to explore the clinical value of these biomarkers in SCCHN. These results also warrant further investigation in understanding if these potential markers of efficacy are strictly related to PI3K inhibitors or might also apply for other therapies.

Disclosure of Potential Conflicts of Interest

L. Lictira is a consultant/advisory board member for AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Debiopharm, Eisai, Merck-Serono, MSD, Novartis, Roche, and SOBI. M. Chol is biomarker clinical manager at Novartis. S. Faivre is a consultant/advisory board member for Bayer, Bristol-Myers Squibb, Eli Lilly, Ipsen, Merck Serono, and Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Chol, N. Solovieff, S. Faivre
Development of methodology: D. Soulières, N. Solovieff, S. Faivre
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Soulières, R. Mesía, É. Remenár, S.-H. Li, A. Karpenko, D. Sellami
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Soulières, R. Mesía, M. Chol, Y.A. Wang, N. Solovieff, D. Sellami, S. Faivre
Writing, review, and/or revision of the manuscript: D. Soulières, L. Lictira, R. Mesía, É. Remenár, M. Chol, N. Solovieff, L. Bourdeau, D. Sellami, S. Faivre
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): É. Remenár, N. Solovieff
Study supervision: D. Soulières, R. Mesía, M. Chol, L. Bourdeau, D. Sellami, S. Faivre

Acknowledgments

The BERIL-1 study was funded by Novartis. We thank the patients who took part in this trial, the investigators, study nurses and clinical research associates from the individual trial centers who supported this trial and Amol Hosing (Novartis Healthcare Pvt. Ltd.) for providing medical editorial assistance with this article.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 26, 2017; revised January 17, 2018; accepted February 22, 2018; published first February 28, 2018.

References

1. Beck TN, Golemis EA. Genomic insights into head and neck cancer. Cancer Head Neck 2016;1:1.
2. Isaacsson Velho PH, Castro G Jr, Chung CH. Targeting the PI3K pathway in head and neck squamous cell carcinoma. Am Soc Clin Oncol Educ Book 2015:123–8.
3. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
4. Cassell A, Grandis JR. Investigational EGFR-targeted therapy in head and neck squamous cell carcinoma. Expert Opin Investig Drugs 2010; 19:709–22.
5. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011;11:9–22.
6. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. J Clin Invest 2012;122:1951–7.
7. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517:576–82.
8. Iglesias-Bartolome R, Martin D, Gutkind JS. Exploiting the head and neck cancer oncogenome: widespread PI3K-mTOR pathway alterations and novel molecular targets. Cancer Discov 2013;3:722–5.
9. Lui VW, Hedberg ML, Li H, Vangara BS, Pendleton K, Zeng Y, et al. Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. Cancer Discov 2013;3:761–9.
10. Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. Cancer Discov 2013;3:770–81.
11. Lee JI, Soria JC, Hassan KA, El-Naggar AK, Tang X, Liu DD, et al. Loss of PTEN expression as a prognostic marker for tongue cancer. Arch Otolaryngol Head Neck Surg 2001;127:1441–5.
12. Squarize CH, Castilho RM, Santos Pinto D Jr. Immunohistochemical evidence of PTEN in oral squamous cell carcinoma and its correlation with the histological malignancy grading system. J Oral Pathol Med 2002; 31:379–84.
13. Bauman JE, Arias-Pulido H, Lee SJ, Fekrazad MH, Ozawa H, Fertig E, et al. A phase II study of temsirolimus and erlotinib in patients with recurrent and/or metastatic, platinum-refractory head and neck squamous cell carcinoma. Oral Oncol 2013;49:461–7.
14. Jimeno A, Bauman JE, Weissman C, Adkins D, Schnadig I, Beauregard P, et al. A randomized, phase 2 trial of docetaxel with or without PX-866, an irreversible oral phosphatidylinositol 3-kinase inhibitor, in patients with relapsed or metastatic head and neck squamous cell cancer. Oral Oncol 2015;51:383–8.
15. Du F, Wu X, Liu Y, Wang T, Qi X, Mao Y, et al. Acquisition of paclitaxel resistance via PI3Kdependent epithelial–mesenchymal transition in A2780 human ovarian cancer cells. Oncol Rep 2013;30:1113–8.
16. Li Y, Chen K, Li L, Li R, Zhang J, Ren W. Overexpression of SOX2 is involved in paclitaxel resistance of ovarian cancer via the PI3K/Akt pathway. Tumour Biol 2015;36:9823–8.
17. Liu Z, Zhu G, Getzenberg RH, Veltri RW. The Upregulation of PI3K/Akt and map kinase pathways is associated with resistance of microtubule-targeting drugs in prostate cancer. J Cell Biochem 2015; 116:1341–9.
18. Soulieres D, Faivre S, Mesia R, Remenar E, Li SH, Karpenko A, et al. Buparlisib and paclitaxel in patients with platinum-pretreated recurrent or metastatic squamous cell carcinoma of the head and neck (BERIL-1): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Oncol 2017;18:323–35.
19. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med 2007;357:2552–61.
20. Bradford CR, Zhu S, Ogawa H, Ogawa T, Ubell M, Narayan A, et al. P53 mutation correlates with cisplatin sensitivity in head and neck squamous cell carcinoma lines. Head Neck 2003;25:654–61.
21. Osman AA, Neskey DM, Katsonis P, Patel AA, Ward AM, Hsu TK, et al. Evolutionary action score of TP53 coding variants is predictive of platinum response in head and neck cancer patients. Cancer Res 2015; 75:1205–15.
22. Lavarino C, Pilotti S, Oggionni M, Gatti L, Perego P, Bresciani G, et al. p53 gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma. J Clin Oncol 2000;18:3936–45.
23. Mroz EA, Rocco JW. Functional p53 status as a biomarker for chemotherapy response in oral-cavity cancer. J Clin Oncol 2010;28:715–7.
24. Perrone F, Bossi P, Cortelazzi B, Locati L, Quattrone P, Pierotti MA, et al. TP53 mutations and pathologic complete response to neoadjuvant cisplatin and fluorouracil chemotherapy in resected oral cavity squamous cell carcinoma. J Clin Oncol 2010;28:761–6.
25. Friedman JM, Stavas MJ, Cmelak AJ. Clinical and scientific impact of human papillomavirus on head and neck cancer. World J Clin Oncol 2014;5:781–91.
26. Feldman R, Gatalica Z, Knezetic J, Reddy S, Nathan CA, Javadi N, et al. Molecular profiling of head and neck squamous cell carcinoma. Head Neck 2016;38(Suppl 1):E1625–38.
27. Lajer CB, von Buchwald C. The role of human papillomavirus in head and neck cancer. APMIS 2010;118:510–9.
28. Partlova S, Boucek J, Kloudova K, Lukesova E, Zabrodsky M, Grega M, et al. Distinct patterns of intratumoral immune cell infiltrates in patients with HPV-associated compared to non-virally induced head and neck squamous cell carcinoma. Oncoimmunology 2015;4:e965570.
29. Fakhry C, Zhang Q, Nguyen-Tan PF, Rosenthal D, El-Naggar A, Garden AS, et al. Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. J Clin Oncol 2014;32: 3365–73.
30. Argiris A, Li S, Ghebremichael M, Egloff AM, Wang L, Forastiere AA, et al. Prognostic significance of human papillomavirus in recurrent or metastatic head and neck cancer: an analysis of Eastern Cooperative Oncology Group trials. Ann Oncol 2014;25:1410–6.
31. Balermpas P, Michel Y, Wagenblast J, Seitz O, Weiss C, Rodel F, et al. Tumour-infiltrating lymphocytes predict response to definitive chemoradiotherapy in head and neck cancer. Br J Cancer 2014;110:501–9.
32. Balermpas P, Rodel F, Rodel C, Krause M, Linge A, Lohaus F, et al. CD8+ tumour-infiltrating lymphocytes in relation to HPV status and clinical outcome in patients with head and neck cancer after postoperative chemoradiotherapy: A multicentre study of the German cancer consortium radiation oncology group (DKTK-ROG). Int J Cancer 2016;138:171–81.

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

33. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med 2016;375:1856–67.
34. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015;27: 450–61.
35. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–8.
36. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909–20.
37. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189–99.
38. Cohen RB. Current challenges and clinical investigations of epidermal growth factor receptor (EGFR)- and ErbB family-targeted agents in the treatment of head and neck squamous cell carcinoma (HNSCC). Cancer Treat Rev 2014;40:567–77.
39. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008; 14:985–90.
40. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31: 1023–31.
41. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45(D1):D777–D83.
42. Seiwert TY, Zuo ZX, Keck MK, Khattri A, Pedamallu CS, Stricker T, et al. Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas. Clin Cancer Res 2015;21:632–41.
43. Singh B, Reddy PG, Goberdhan A, Walsh C, Dao S, Ngai I, et al. p53 regulates cell survival by inhibiting PIK3CA in squamous cell carcinomas. Genes Dev 2002;16:984–93.
44. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34.
45. Economopoulou P, Perisanidis C, Giotakis EI, Psyrri A. The emerging role of immunotherapy in head and neck squamous cell carcinoma (HNSCC): anti-tumor immunity and clinical applications. Ann Transl Med 2016; 4:173.
46. Mandal R, Senbabaoglu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight 2016;1:e89829.
47. Nguyen N, Bellile E, Thomas D, McHugh J, Rozek L, Virani S, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. Head Neck 2016;38:1074–84.
48. Karpathiou G, Casteillo F, Giroult JB, Forest F, Fournel P, Monaya A, et al. Prognostic impact of immune microenvironment in laryngeal and pharyngeal squamous cell carcinoma: Immune cell subtypes, immuno-suppressive pathways and clinicopathologic characteristics. Oncotarget 2017; 8:19310–22.
49. Zhao T, Li C, Wu Y, Li B, Zhang B. Prognostic value of PD-L1 expression in tumor infiltrating immune cells in cancers: A meta-analysis. PLoS One 2017;12:e0176822.
50. Ali K, Soond DR, Pineiro R, Hagemann T, Pearce W, Lim EL, et al. Inactivation of PI(3)K p110delta breaks regulatory T-cell-mediated immune tolerance to cancer. Nature 2014;510:407–11.
51. Sai J, Owens P, Novitskiy SV, Hawkins OE, Vilgelm AE, Yang J, et al. PI3K Inhibition Reduces Mammary Tumor Growth and Facilitates Antitumor Immunity and Anti-PD1 Responses. Clin Cancer Res 2017; 23:3371–84.

Published OnlineFirst February 28, 2018; DOI: 10.1158/1078-0432.CCR-17-2644

Clinical Cancer Research

Molecular Alterations and Buparlisib Efficacy in Patients with Squamous Cell Carcinoma of the Head and Neck: Biomarker Analysis from BERIL-1

Denis Soulières, Lisa Licitra, Ricard Mesía, et al.

Clin Cancer Res 2018;24:2505-2516. Published OnlineFirst February 28, 2018.