The KRAS-Variant and Cetuximab Response in Head and Neck Squamous Cell Cancer
A Secondary Analysis of a Randomized Clinical Trial

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IMPORTANCE There is a significant need to find biomarkers of response to radiotherapy and cetuximab in locally advanced head and neck squamous cell carcinoma (HNSCC) and biomarkers that predict altered immunity, thereby enabling personalized treatment.

OBJECTIVES To examine whether the Kirsten rat sarcoma viral oncogene homolog (KRAS)-variant, a germline mutation in a microRNA-binding site in KRAS, is a predictive biomarker of cetuximab response and altered immunity in the setting of radiotherapy and cisplatin treatment and to evaluate the interaction of the KRAS-variant with p16 status and blood-based transforming growth factor β1 (TGF-β1).

DESIGN, SETTING, AND PARTICIPANTS A total of 891 patients with advanced HNSCC from a phase 3 trial of cisplatin plus radiotherapy with or without cetuximab (NRG Oncology RTOG 0522) were included in this study, and 413 patients with available samples were genotyped for the KRAS-variant. Genomic DNA was tested for the KRAS-variant in a CLIA-certified laboratory. Correlation of the KRAS-variant, p16 positivity, outcome, and TGF-β1 levels was evaluated. Hazard ratios (HRs) were estimated with the Cox proportional hazards model.

MAIN OUTCOMES AND MEASURES The correlation of KRAS-variant status with cetuximab response and outcome, p16 status, and plasma TGF-β1 levels was tested.

RESULTS Of 891 patients eligible for protocol analyses (786 male [88.2%], 105 [11.2%] female, 810 white [90.9%], 81 nonwhite [9.1%]), 413 had biological samples for KRAS-variant testing, and 376 had plasma samples for TGF-β1 measurement. Seventy patients (16.9%) had the KRAS-variant. Overall, for patients with the KRAS-variant, cetuximab improved both progression-free survival (PFS) for the first year (HR, 0.31; 95% CI, 0.10-0.94; P = .04) and overall survival (OS) in years 1 to 2 (HR, 0.19; 95% CI, 0.04-0.86; P = .03). There was a significant interaction of the KRAS-variant with p16 status for PFS in patients treated without cetuximab. The p16-positive patients with the KRAS-variant treated without cetuximab had worse PFS than patients without the KRAS-variant (HR, 2.59; 95% CI, 0.91-7.33; P = .07). There was a significant 3-way interaction among the KRAS-variant, p16 status, and treatment for OS (HR, for KRAS-variant, cetuximab and p16 positive, 0.22; 95% CI, 0.03-1.66; HR for KRAS-variant, cetuximab and p16 negative, 1.43; 95% CI, 0.48-4.26; HR for KRAS-variant, no cetuximab and p16 positive, 2.48; 95% CI, 0.64-9.65; and HR for KRAS-variant, no cetuximab and p16 negative, 0.61; 95% CI, 0.23-1.59; P = .02). Patients with the KRAS-variant had significantly elevated TGF-β1 plasma levels (median, 23 376.49 vs 18 476.52 pg/mL; P = .03) and worse treatment-related toxic effects.

CONCLUSIONS AND RELEVANCE Patients with the KRAS-variant with HNSCC significantly benefit from the addition of cetuximab to radiotherapy and cisplatin, and there is a significant interaction between the KRAS-variant and p16 status. Elevated TGF-β1 levels in patients with the KRAS-variant suggests that cetuximab may help these patients by overcoming TGF-β1–induced suppression of antitumor immunity.

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he current standard of care for the nonsurgical management of locally advanced head and neck squamous cell carcinoma (HNSCC) is concurrent platinum-based chemotherapy and radiotherapy. Unfortunately, however, this approach results in an approximately 50% treatment failure rate in unselected patients.1,2 Cetuximab is an anti-epidermal growth factor receptor (EGFR) monoclonal antibody previously reported to be beneficial for such patients, improving survival when given with definitive radiotherapy or with cisplatin and fluorouracil in recurrent or metastatic HNSCC.3,4 However, a randomized phase 3 clinical trial (NRG Oncology RTOG 0522) failed to show any improvement in survival with the addition of 8 weeks of cetuximab to cisplatin and radiotherapy.2 To date, beyond EGFR surface expression,2 no predictive biomarker of cetuximab response has been identified for these patients.

Recently, human papillomavirus, reliably measured by p16 expression, has been established as a prognostic biomarker in HNSCC, with p16-positive patients having a favorable outcome compared with p16-negative patients.1,5,7 One reason for the better prognosis in p16-positive disease is thought to be attributable to immune-mediated mechanisms because cisplatin and radiotherapy expose p16-associated antigens, which are immunogenic,8 enabling host-mediated immune tumor killing. Consistent with a prior report,2 p16-positive patients in NRG Oncology RTOG 0522 had improved outcome compared with p16-negative patients. However, there was no benefit of cetuximab treatment when p16 status was considered in NRG Oncology RTOG 0522. One hypothesis put forward is lower EGFR tumor cell staining in p16-positive tumors in NRG Oncology RTOG 0522 vs p16-negative tumors (22% vs 30%).2

Beyond inhibition of EGFR signaling, however, cetuximab has also been found to work through immune-mediated mechanisms by enhancing adaptive immunity via antibody-dependent cell-mediated cytotoxicity9,10 and by better enabling cytotoxic T-lymphocyte cross-priming by dendritic cells.12 Recently, cetuximab combined with chemotherapy was further confirmed to trigger immunogenic cell death in mouse models through increased phagocytosis by dendritic cells.23 These immune-based mechanisms of cetuximab efficacy have not yet been studied in the setting of radiotherapy, although radiotherapy also enables antitumor immunity, with the release of danger signals, and dendritic and T-cell activation.13,15 Recently, a biomarker of altered immune function, FCGR2A, a polymorphism in an Fcy receptor, was found to influence the response rate to cetuximab for patients with Kirsten rat sarcoma viral oncogene homolog (KRAS) (OMIM 190070)-mutant tumors,16 indicating that inherited polymorphisms can affect cetuximab response.

In colon cancer, response to cetuximab is associated with an inherited biomarker disrupting KRAS, referred to as the KRAS-variant.17 The KRAS-variant is the first microRNA-binding site mutation discovered in cancer and is a functional germline mutation in the 3’ untranslated region of KRAS. Although the KRAS-variant is rare in tumors with acquired KRAS mutations,18,19 it leads to gene expression patterns consistent with KRAS addiction across tumor types.19,20 The KRAS-variant is associated with increased cancer risk18-20 and the development of multiple cancers21,22 and more recently was found to be a predictive biomarker of response to cancer treatment irrespective of tumor type or tumor-acquired mutations.23-28 In HNSCC, the KRAS-variant is found in 15% to 32% of patients29,30 and is therefore highly relevant. Patients with HNSCC and the KRAS-variant have previously been reported to have a poor response to platinum therapy, similar to findings for patients with the KRAS-variant and other cancer types.23,29-31

Although the mechanisms leading to the predictive power of the KRAS-variant in treatment response have not been fully elucidated, one could hypothesize that the KRAS-variant identifies patients with altered immunity based on the fact that KRAS signaling and let-7 microRNA expression, both universally abnormal in KRAS-variant–associated tumors,19,23,29 are critical in systemic immunity.32,33 In addition, mutant KRAS directly inhibits antitumor immunity through induction of transforming growth factor β1 (TGF-β1) secretion, which is known to lead to conversion of conventional T cells into regulatory T cells or type 17 helper T cells.34-36 In the setting of radiotherapy, TGF-β1 is proposed to be a master regulator of treatment response, with high levels preventing radiation-induced antitumor immunity.37 Interestingly, in cells with high TGF-β1 levels, cetuximab has been found to restore the killing activity of patient natural killer cells against primary HNSCC cells via enhanced antibody-dependent cell-mediated cytotoxicity in a dose-dependent manner.38 Cetuximab response has also been found to overcome TGF-β1–expressing regulatory T cells, known to inhibit natural killer cell killing,39 providing a potential mechanism of action. To date, the role of altered immunity and TGF-β1 levels as potential mechanisms of cetuximab response in patients with the KRAS-variant has not been evaluated.

Thus, we sought to test the predictive role of the KRAS-variant for outcomes in patients with HNSCC in the setting of radiotherapy, cisplatin, and cetuximab, considering p16 status. We further evaluated immunity in patients with the KRAS-variant through plasma TGF-β1 levels.

**Methods**

**Protocol and Patients**

NRG Oncology RTOG 0522 was a phase 3 trial that tested the addition of cetuximab to radiotherapy with concurrent cisplatin...
for patients with advanced HNSCC.2 The study was approved by all institutional review boards where patients were enrolled, and oral informed consent was obtained from all study participants. Race and sex data were collected and are reported in eTable 1 in the Supplement. Eligible patients had pathologically proven squamous cell carcinoma of the oropharynx, hypopharynx, or larynx, with selected stage III or IV disease (T2N2-3MO or T3-4NMO [any N]), Zubrod performance status of 0 to 1, age of 18 years or older, and adequate bone marrow, hepatic, and renal function. Human papillomavirus status was evaluated by p16 expression as previously described.1,2 A total of 940 patients were enrolled into NRG Oncology RTOG 0522, of whom 891 (94.8%) were eligible for protocol analyses. A total of 413 of 891 patients had biological samples available for KRAS-variant analysis (46.4%) (eFigure 1 in the Supplement). Patients not tested for the KRAS-variant had significantly younger age (median, 56 vs 58; P = .02) than patients tested for the KRAS-variant, but the difference in medians was only 2 years (56 vs 58 years) (eTable 1 in the Supplement). Progression-free survival (PFS) and overall survival (OS) were similar for the patients tested and not tested for the KRAS-variant (PFS hazard ratio [HR], 0.92; 95% CI, 0.76-1.13; P = .44; OS HR, 0.99; 95% CI, 0.78-1.25; P = .93). DNA genotyping was performed from January 2015 to May 2015. At the time of analysis, median follow-up for surviving patients was 4.8 years (range, 0.2-6.9 years). Of the 413 study participants tested for the KRAS-variant, 5 (1.2%) were homozygous (GG) and 65 (15.7%) were heterozygous (TG), resulting in an overall prevalence of 16.9%. The prevalence of the KRAS-variant was not significantly different in p16-negative (17.4%) vs p16-positive (16.0%) patients, in agreement with a prior study.30

Of the 70 patients with the KRAS-variant, the cetuximab-treated subset had fewer patients of white race (28 [87.5%] of 32 vs 38 [100%] of 38, P = .04). Within the nonvariant cohort, the cetuximab-treated subset had a significantly younger age than the subset not receiving cetuximab, but the difference in medians was only 2 years (59 vs 57 years, 174 cetuximab treated vs 169 not cetuximab treated, P = .05) (eTable 2 in the Supplement).

**KRAS-Variant Testing**

Genomic DNA from peripheral blood mononuclear cells or whole blood was isolated as previously described,40 and 100 ng was analyzed in a Clinical Laboratory Improvement Amendments–certified laboratory for the KRAS-variant (MiraDx). Patients that were homozygous (GG) were grouped with those that were heterozygous (TG) for these analyses.

**Plasma TGF-β1 Levels**

Plasma levels of TGF-β1 from 376 patients enrolled in NRG Oncology RTOG 0522 were measured using multiplex bead assays with the use of reagents from Millipore and the Luminex 100 system as previously described.41 The assay was run using manufacturer controls to ensure precision. The correlation between KRAS-variant status and plasma TGF-β1 levels was evaluated by Wilcoxon rank sum test.

**Statistical Analysis**

Locoregional failure (LRF), distant metastasis (DM), PFS, and OS were as defined in the NRG Oncology RTOG 0522 protocol. The LRF and DM rates were estimated by the cumulative incidence method.42 The PFS and OS rates were estimated by the Kaplan-Meier method.43 The HRs were estimated by the Cox proportional hazards model.44 Adverse events were graded by Common Terminology Criteria for Adverse Events, version 3.0. Odds ratios (ORs) were estimated by logistic regression. Patient characteristics were compared with the Fisher exact test (categorical variables) or Wilcoxon rank sum test (ordinal or continuous variables). All analyses were performed using SAS statistical software, version 9.4 (SAS Institute Inc). P < .05 was considered statistically significant.

**Results**

**Prediction of Response to Cetuximab**

Of 891 patients eligible for protocol analyses (786 male [88.2%], 105 [11.2%] female, 810 white [90.9%], 81 nonwhite [9.1%]), 413 had biological samples for KRAS-variant testing, and 376 had plasma for TGF-β1 measurement. We investigated the association of the KRAS-variant with cetuximab response in HNSCC and found a significant, positive effect of 8 weeks of cetuximab treatment for all patients with the KRAS-variant. Patients with the KRAS-variant had significantly improved PFS in the first year (HR, 0.31; 95% CI, 0.12-0.94; P = .04) but not thereafter (HR, 1.76; 95% CI, 0.62-4.95; P = .28) (Figure 1A). The cetuximab treatment effect significantly varied over time (P = .02 for interaction between treatment and PFS time [>1 year]). In the nonvariant group, there was no effect of cetuximab on PFS with a treatment effect HR (cetuximab vs no cetuximab) of 1.00 (95% CI, 0.72-1.38; P = .98). In multivariate analysis, the interaction among treatment, time (>1 year), and KRAS-variant group remained significant (HR, 0.42; 95% CI, 0.14-1.26, for early treatment effect; HR, 1.22; 95% CI, 0.53-2.80, for late treatment effect; P = .02), indicating that there is an interaction between treatment and time in the KRAS-variant group (Table I). The pattern of failure in a multivariate analysis suggested that DM rather than LRF may be a more likely contributor to the difference in PFS for patients with the KRAS-variant: in the KRAS-variant group, the treatment effect is 0.45 (95% CI, 0.12-1.70) for DM and 0.84 (95% CI, 0.29-2.42) for LRF (eFigure 2 and eFigure 3 in the Supplement). In the nonvariant group, the treatment effects are 0.90 (95% CI, 0.48-1.70) for DM and 1.24 (95% CI, 0.80-1.92) for LRF.

Patients with the KRAS-variant also had significantly improved OS in the first 2 years (HR, 0.19; 95% CI, 0.04-0.86; P = .03) but not thereafter (HR, 2.34; 95% CI, 0.58-9.41; P = .23) (Figure 1B). Again, the cetuximab treatment effect significantly varied over time (P = .02 for interaction between treatment effect and survival time [>2 years]). In the nonvariant group, cetuximab treatment had no effect on OS (treatment effect HR [cetuximab vs no cetuximab], 0.90; 95% CI, 0.62-1.30; P = .56). In a multivariate analysis, the interaction among treatment, time (>2 years), and KRAS-variant group remained significant (Table I), indicating that there is an in-
interaction between treatment and time in the KRAS-variant group. The treatment effect in the first 2 years for the KRAS-variant group is 0.27 (95% CI, 0.06-1.21; \( P = .09 \)) and 1.47 (95% CI, 0.53-4.03; \( P = .46 \)) thereafter (\( P = .05 \) for the interaction).

**Interaction With p16 Status for PFS**

Because p16, a key cell cycle inhibitor, is a known prognostic biomarker in HNSCC, we evaluated outcome in patients with the KRAS-variant while considering p16 status, without or with cetuximab treatment. For PFS, in patients treated without cetuximab, the 2-way interaction between KRAS and p16 was significant (Table 2). The p16-positive patients with the KRAS-variant treated without cetuximab had worse PFS than the p16-positive patients without the KRAS-variant (HR, 2.59; 95% CI, 0.91-7.33; \( P = .07 \)) (Figure 2A). However, in cetuximab-treated patients, there was no longer a difference in outcome between the p16-positive patients with the KRAS-variant vs without the KRAS-variant (HR, 0.89; 95% CI, 0.34-2.35; \( P = .82 \)) (Figure 2A).

**Interaction With p16 and Treatment for OS**

For OS, there is a significant 3-way interaction among cetuximab treatment, KRAS-variant status, and p16 status. There is
also a significant 2-way interaction between treatment and KRAS for p16-positive patients and between treatment and p16 status for patients with the KRAS-variant (Table 2). In patients treated without cetuximab, p16-positive patients with the KRAS-variant had worse OS than p16-positive patients without the KRAS-variant (HR, 2.48; 95% CI, 0.64-9.65; \( P = .19 \)), which appeared to be nonsignificantly reversed with cetuximab treatment (HR, 0.22; 95% CI, 0.03-1.66; \( P = .14 \)) (Figure 2B). In p16-positive patients, the cetuximab treatment effect is 0.21 (95% CI, 0.02-2.04; \( P = .18 \)) for patients with the KRAS-variant compared with 2.36 (95% CI, 0.98-5.67; \( P = .05 \)) for patients without the KRAS-variant.

### Table 1. Multivariate Analysis of PFS and OS on the Basis of KRAS-Variant Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>PFS (n = 116 Events)</th>
<th>OS (n = 85 Events)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)*</td>
<td>( P ) Value</td>
</tr>
<tr>
<td>KRAS-variant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.42 (0.14-1.26)</td>
<td>.12</td>
</tr>
<tr>
<td>Late</td>
<td>1.22 (0.53-2.80)</td>
<td>.64</td>
</tr>
<tr>
<td>Nonvariant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>1.20 (0.80-1.80)</td>
<td>.39</td>
</tr>
<tr>
<td>Late</td>
<td>0.79 (0.47-1.33)</td>
<td>.38</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

* The HRs were estimated from Cox proportional hazards models, including treatment (cetuximab vs no cetuximab), treatment × PFS and OS time interaction, KRAS (variant vs nonvariant), treatment × KRAS interaction, treatment × PFS and OS time × KRAS interaction, age, Zubrod performance status (1 vs 0), primary site (oropharynx vs others), T stage (T4 vs T2-3), and N stage (N2b-3 vs N0-2a).

b First year for PFS and first 2 years for OS.

### Table 2. Multivariate Analysis of PFS and OS on the Basis of p16, Cetuximab, and KRAS-Variant Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>PFS (n = 116 Events)</th>
<th>OS (n = 85 Events)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)*</td>
<td>( P ) Value</td>
</tr>
<tr>
<td>Treatment × KRAS</td>
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<td></td>
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<tr>
<td>× p16</td>
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<td>.20</td>
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<tr>
<td>If p16-positive</td>
<td>NA</td>
<td>.14</td>
</tr>
<tr>
<td>Treatment × p16</td>
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<td></td>
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<tr>
<td>If KRAS-variant</td>
<td>NA</td>
<td>.51</td>
</tr>
<tr>
<td>If KRAS-nonvariant</td>
<td>NA</td>
<td>.12</td>
</tr>
<tr>
<td>KRAS × p16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If cetuximab</td>
<td>NA</td>
<td>.84</td>
</tr>
<tr>
<td>If no cetuximab</td>
<td>NA</td>
<td>.04</td>
</tr>
<tr>
<td>Treatment effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If KRAS-variant and p16 positive</td>
<td>0.60 (0.17-2.10)</td>
<td>.42</td>
</tr>
<tr>
<td>If KRAS nonvariant and p16 positive</td>
<td>1.74 (0.88-3.42)</td>
<td>.11</td>
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<tr>
<td>KRAS effect</td>
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<tr>
<td>If no cetuximab and p16 positive</td>
<td>2.59 (0.91-7.33)</td>
<td>.07</td>
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<tr>
<td>If cetuximab and p16 positive</td>
<td>0.89 (0.34-2.35)</td>
<td>.82</td>
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<td>p16 effect</td>
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<td>If cetuximab and KRAS-variant</td>
<td>0.73 (0.20-2.73)</td>
<td>.64</td>
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<tr>
<td>If cetuximab and KRAS nonvariant</td>
<td>0.63 (0.36-1.11)</td>
<td>.11</td>
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<tr>
<td>If no cetuximab and KRAS-variant</td>
<td>1.34 (0.40-4.44)</td>
<td>.64</td>
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<tr>
<td>If no cetuximab and KRAS nonvariant</td>
<td>0.32 (0.17-0.61)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; NA, not applicable (HRs for interaction terms are not meaningful and are therefore not reported); OS, overall survival; PFS, progression-free survival.

* The HRs were estimated from Cox proportional hazards models, including treatment (cetuximab vs no cetuximab), treatment × PFS and OS time interaction, KRAS (variant vs nonvariant), treatment × KRAS interaction, treatment × PFS and OS time × KRAS interaction, age, Zubrod performance status (1 vs 0), primary site (oropharynx vs others), T stage (T4 vs T2-3), and N stage (N2b-3 vs N0-2a).

Immunologic Landscape and Toxic Effects in Patients With the KRAS-Variant

On the basis of the differential effect of cetuximab, especially in p16-positive patients, we investigated immune factors in patients with the KRAS-variant. We measured plasma TGF-β1 (generally a negative regulator of local immune response) levels in patients from NRG Oncology RTOG 0522 and found that patients with the KRAS-variant had significantly higher plasma TGF-β1 levels (median, 23,376.49 pg/mL; interquartile range, 12,574.03-44,809.10 pg/mL; range, 5034.35-109,758.72 pg/mL) compared with patients without the KRAS-variant (median, 18,477 pg/mL; interquartile range, 8837.00-32,378.43 pg/mL; range, 2261.42-123,264.70 pg/mL) (Wilcoxon rank sum test,
To further evaluate whether KRAS-variant and p16 status (eTable 3 in the Supplement). A sex expected, both patients with KRAS-variant status and cetuximab treatment, OR, 0.82; 95% CI, 0.23-2.89; P = .76). In contrast, in patients without the KRAS-variant, the addition of cetuximab significantly increased grade 3 to 4 skin reactions inside the portal (11.2% vs 21.8%; OR, 2.21; 95% CI, 1.21-4.01; P = .05), but again a test of interaction was not significant (P = .16) (eTable 5 in the Supplement). As expected, both patients with and without the KRAS-variant developed increased skin reaction outside the portal with cetuximab (nonvariant OR, 50.15; 95% CI, 6.81-369.54; P < .001; KRAS-variant OR, 8.54; 95% CI, 0.97-75.20; P = .05) (eTable 6 in the Supplement).

To further evaluate whether KRAS-variant status and TGF-β1 levels together predict toxic effects, we evaluated patients with the KRAS-variant with known TGF-β1 levels (n = 65)
and looked at grade 3 to 4 mucositis or in-field skin toxic effects. We only included patients not treated with cetuximab because cetuximab is known to be a radiosensitizer. We found that patients with the KRAS-variant with TGF-β1 levels greater than vs at the median or below treated without cetuximab appeared to have an increase in risk of grade 3 to 4 toxic effects (OR, 2.31; 95% CI, 0.57-9.41). However, the interaction between the KRAS-variant and TGF-β1 was not significant (P = .40). In contrast, patients without the KRAS-variant with TGF-β1 levels greater than vs at the median or below treated without cetuximab had little correlation of TGF-β1 levels on toxic effects (OR, 1.19; 95% CI, 0.62-2.25).

**Discussion**

Patients with advanced HNSCC with the KRAS-variant significantly benefit from the addition of cetuximab to radiotherapy and cisplatin therapy, resulting in improved PFS and OS. We found that this benefit fades over time, possibly because of the short course of cetuximab delivered in this study. We further found that there is a significant interaction among the KRAS-variant, p16 status, and cetuximab, with p16-positive patients with the KRAS-variant doing poorly compared with p16-positive patients without the KRAS-variant, a deficit that seems to be overcome by cetuximab. Furthermore, we found that patients with the KRAS-variant have significantly elevated TGF-β1 levels, suggesting that they may have a baseline immunodeficient and/or anti–tumor immune state, which cetuximab in combination with radiotherapy may be helping to overcome.

For HNSCC, although p16 is an important prognostic biomarker, it appears that knowledge of p16 status and KRAS-variant status may be important to determine optimal personalized treatment for these patients. It is particularly notable that p16-positive patients with the KRAS-variant had the worst outcomes of all subgroups of patients with HNSCC in our study, which appeared to be improved with a short course of cetuximab treatment given with radiotherapy and cisplatin. These patients should perhaps be considered a different entity from p16-positive patients without the KRAS-variant, who had excellent outcomes with radiotherapy and cisplatin alone. Work is ongoing to further validate these observations.

Our finding that patients with HNSCC and the KRAS-variant may have an altered immune state compared with patients without the KRAS-variant is novel but perhaps not surprising, considering that the KRAS-variant is germline and, thus, present in all cells, including immune cells. This inherited mutation is known to lead to globally altered gene expression profiles consistent with KRAS addiction as well as altered expression of the let-7 microRNA, both of which have significant effects on the immune system. In addition, our findings of elevated TGF-β1 expression in these patients, which has long been known to be involved in immunity and immunosuppression, further supports our hypothesis that patients with the KRAS-variant are immunosuppressed. Baseline differences in immunity could also help explain the increased cancer risk found in patients with the KRAS-variant. Interestingly, cetuximab overcomes the impaired natural killer group 2D–dependent functionality of activated natural killer cells in the presence of TGF-β1, potentially explaining the global benefit of cetuximab treatment seen in patients with the KRAS-variant. Whether the altered TGF-β1 we see in these patients represents baseline perturbations in homeostasis or altered responses to tumor challenge and is a finding that will be found for all KRAS-variant patients with cancer remains unclear and is the subject of important ongoing investigations.

Although the sample size was small in this study and a larger study would strengthen the results, our current findings are consistent with prior work with KRAS-variant patients with recurrent or metastatic HNSCC treated with cisplatin with or without cetuximab, revealing that the addition of cetuximab improved median survival to 3.9 months; however, the interaction with p16 status could not be evaluated previously because of the small sample size. Our findings are also consistent with those found in KRAS-variant patients with colon cancer, in whom cetuximab monotherapy is clearly beneficial. What this study has additionally found is the potential importance of cetuximab delivered in conjunction with radiotherapy for patients with the KRAS-variant to seemingly enable them to mount appropriate immunologic responses after tumor-targeted irradiation, diminishing metastatic failures. We also find that patients with the KRAS-variant may have baseline normal tissue radiation sensitivity, possibly through their elevated TGF-β1 levels. Although cetuximab is a known radiosensitizer, there did not appear to be worsened radiosensitivity with the addition of cetuximab for patients with the KRAS-variant. Studies are ongoing to better understand the mechanisms of radiosensitivity in these patients to further personalize their treatment.

**Limitations**

This was an ad hoc analysis of a prospective study with a limited number of KRAS-variant patients. Additional prospective trials are planned to directly confirm the findings. End points to be further validated include the findings that the KRAS-variant identifies patients with altered immunity and differential sensitivity to radiotherapy and immune-modulating therapies.

**Conclusions**

Although a germline biomarker that can identify individuals with altered immunity that can be used to direct the best therapeutic combinations is a bold new concept, the KRAS-variant is unfolding as a true candidate. On the basis of the findings of this study, defining how anticancer agents affecting the tumor-host-immune association differently affect patients with the KRAS-variant is a critical and pressing need because this knowledge could help in personalizing developing immune-based cancer therapies for all patients.
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REFERENCES


The KRAS Variant as a Predictive Biomarker of Cetuximab Response in Head and Neck Cancer

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Invited Commentary

In their interesting report, Weidhaas et al\(^1\) posit that a germ-line mutation in the 3′ untranslated region of the KRAS variant is a predictive marker of improved response to cetuximab for patients with locally advanced head and neck squamous cell carcinoma (HNSCC). While the results presented therein are intriguing, we would advise caution regarding their interpretation. The study by Weidhaas et al has several strengths; first, being focused on a well-characterized germline polymorphism that has been previously described as a predictive biomarker in a similar context for colon cancer.\(^2\) The evaluation of a single marker increases statistical power by limiting the number of comparisons performed;\(^3\) however, while only 1 variant was analyzed, multiple hypotheses were evaluated, thereby increasing the potential for inflated type I error.\(^3\)

A second strength was the use of samples from patients enrolled in a randomized clinical trial, which is a recommended approach to identifying predictive biomarkers.\(^4\) In this study, however, only a subset (413 out of 891) of the randomized participants had available material for genotyping and were therefore subjects of this analysis. This nonrandom inclusion criterion (ie, availability of tissue) rendered this study prone to potential biases. Moreover, while the samples emanated from a randomized clinical trial, patients were not stratified based on their KRAS variant genotype prior to randomization, in a so-called “interaction” design, which is the ideal approach when evaluating the predictive value of a potential biomarker.\(^4\) Indeed, the authors reported a number of potential confounders including KRAS-variant subjects being significantly younger, and significantly fewer white patients with the KRAS variant were treated with cetuximab. Moreover, it...