original reports

HRAS Mutations Define a Distinct Subgroup in Head and Neck Squamous Cell Carcinoma

Niamh Coleman, MBBCH, PhD[1]; Kathrina L. Marcelo, PhD[2]; Julia F. Hopkins[3]; Nusrat Israr Khan, PhD[2]; Robyn Du[2];
Lingzhi Hong, MD, PhD[2]; Edward Park[4]; Binaifer Balsara[4]; Mollie Leoni[4]; Curtis Pickering, MD, PhD[5]; Jeffrey Myers, MD, PhD[5];
John Heymach, MD, PhD[2]; Lee A. Albacker, PhD[3]; David Hong, MD[1]; Maura Gillison, MD, PhD[2]; and Xiuning Le, MD, PhD[2]

abstract

PURPOSE In head and neck squamous cell carcinoma (HNSCC), *HRAS* mutation is a new actionable oncogene driver. We aimed to evaluate *HRAS* mutational variants, comutation profile, and survival outcomes of this molecularly defined population.

METHODS We leveraged four deidentified patient data sets with *HRAS*-mutant HNSCC, MD Anderson Cancer Center, Kura Oncology, Inc trial, Foundation Medicine, and American Association for Cancer Research GENIE v.12. Patient demographic information and clinical courses were extracted, when available, in addition to *HRAS* mutation type and co-occurring mutations. Survival outcomes were analyzed (Kaplan-Meier method).

RESULTS Two hundred forty-nine patients with *HRAS*-mutant HNSCC were identified from the four data sets. Median age ranged from 55 to 65 years, with a higher frequency in male patients (64%); the majority of *HRAS*-mutant HNSCC occurred in human papillomavirus–negative HNSCC. *HRAS* mutation patterns were similar across data sets; G12S was the most common (29%). Treatment responses to tipifarnib were not codon-specific. Compared with wild-type, significantly co-occurring mutations with *HRAS* were *Casp8* (Fisher's exact test, $P < .00013$), *TERT* ($P < .0085$), and *NOTCH1* ($P < .00013$). Analysis of clinical courses from the MD Anderson Cancer Center and Kura Oncology, Inc data sets demonstrated poor clinical outcomes with a high rate of recurrence following primary definitive treatment (50%-67% relapse < 6 months) and short disease-free survival (4.0 months; 95% CI, 1.0 to 36.0) and overall survival (OS; 15.0 months; 95% CI, 6.0 to 52.0). Use of tipifarnib in this data set demonstrated improved OS (25.5 months; 95% CI, 18.0 to 48.0).

CONCLUSION Oncogenic mutations in *HRAS* occur in 3%-4% of HNSCC, with G12S being the most frequent. Without targeted therapy, patients with *HRAS*-mutant HNSCC had poor clinic outcomes; observable trend toward improvement in OS has been noted in cohorts receiving treatments such as tipifarnib. The comutation pattern of *HRAS*-mutant in HNSCC is distinct, which may provide insight to future therapeutic combination strategies.

BACKGROUND

Head and neck squamous cell carcinoma (HNSCC) are a heterogeneous group of tumors arising from the oral cavity, pharynx, and larynx, with varying etiological factors, traditionally, related primarily to tobacco and alcohol exposure or human papillomavirus (HPV) infection.[1] Despite recent advances using programmed death-1 targeting agents with the standard-of-care chemotherapy and radiation, prognosis remains poor and median overall survival (OS) is 13-15 months.[2]

Activating mutations in the Ras proto-oncogenes (K-, N-, H-) are initiating oncogenic events in human cancer,[3] and the development of Ras-targeted agents has been historically challenging. Recently, a better understanding of Ras signaling has led to the development of promising compounds directly inhibiting *KRAS* G12C mutant tumors.[4] Recent molecular analysis studies have shown that *HRAS* is the predominant mutated RAS isoform in squamous cell carcinomas of several cancers, including HNSCC,[5] where mutations in *HRAS* have been reported to occur in 4%-8%.[6]

All RAS isoforms are farnesyltransferase (FTase) substrates; however, only HRAS is exclusively dependent upon farnesylation. Subsequent clinical evidence confirms that *HRAS*-mutant tumors are susceptible to inhibition of FTase.[7] Tipifarnib, an FTase inhibitor (FTI), is a first-in-class nonpeptidomimetic quinolinone that binds and potently inhibits FTase (half maximal inhibitory concentration of 0.86 nM for lamin B farnesylation).[8] For patients with metastatic or recurrent HNSCC, patients

ASCO

CONTEXT

Key Objective

To evaluate *HRAS* mutational variants, comutation profile, and survival outcomes in head and neck squamous cell carcinoma (HNSCC), we leverage four independent data sets to better define this patient population.

Knowledge Generated

Mutations in oncogenic *HRAS* are rare (3%) in patients with HNSCC and occur more frequently in human papillomavirus (HPV)–negative tumors, with *HRAS* G12S being the most common alteration. Responses to tipifarnib are not specific to *HRAS* mutation codon site. Significantly co-occurring mutations with *HRAS* are *Casp8*, *TERT*, and *NOTCH1*.

Relevance

We confirm the comutation profile of *HRAS*-mutant HNSCC as unique, and understanding the comutation profile could affect future treatment strategies in *HRAS*-mutant HNSCC. We demonstrate that *HRAS* mutations occur in both HPV-positive and HPV-negative HNSCC, although a more focused testing strategy in HPV-negative patients may be important for clinicians in resource-limited settings.

whose tumor harboring *HRAS* mutations, tipifarnib treatment has produced an objective response rate of 55% and a median OS of 15.4 months,[9] which led to the US Food and Drug Administration granting Breakthrough Therapy Designation to tipifarnib for the treatment of patients with recurrent or metastatic *HRAS*-mutant HNSCC.

Here, we leveraged four independent data sets of *HRAS*-mutant HNSCC and aimed to evaluate the mutational profile and co-occurring genetic alteration landscape of *HRAS*-mutant HNSCC from four different data sets: MD Anderson Cancer Center (MDACC), KURA KO-TIP-001 trial participants (Kura Oncology, Inc, Boston, MA [Kura]) Foundation Medicine Inc (FMI)–profiled cases, and American Association for Cancer Research (AACR) Project GENIE v.12.[10] Thus, in this study, we provide the largest genomic data set of *HRAS*-mutant HNSCC reported to date and explore the clinical and genomic landscape of *HRAS*-mutant HNSCC.

METHODS

Data Sets and Patient Population

HRAS-mutant HNSCC cases were collected from four data sets: MDACC, KURA KO-TIP-001 trial participants (ClinicalTrials.gov identifier: NCT02383927), FMI-profiled cases (samples collected from 2013 to 2020), and AACR Project GENIE v.12,[10] an international clinicogenomic data-sharing consortium, now in its twelfth version (analyzed in July 2022). Co-occurring genomic profiles were evaluated in each data set. Please refer Appendix 1 for detail for each data set.

Genomic Profiling

In the FMI cohort, genomic data were collected using a tissue-based, targeted comprehensive genomic profiling assay (FoundationOne or FoundationOneCDx)[11] in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State–approved laboratory (FMI), as previously described. Tumor mutation burden (TMB) is the number of nondriver somatic coding mutations per megabase (mut/Mb) of genome sequenced.[12] TMB high was defined as ≥ 10 mut/Mb. TMB low was defined as < 10 mut/Mb.

In the MDACC data set, tumor samples were evaluated per standard-of-care procedure. All procedures were performed in a Clinical Laboratory Improvement Amendments–compliant environment. For genomic analysis, samples were sequenced in the MDACC molecular diagnostic laboratory using assays, the Oncomine platform (Thermo Fisher Scientific, Waltham, MA) for the detection of somatic mutations in the coding sequence of 143 cancer-related genes, as previously described[13]; the Ion Ampliseq 48-Gene Assay for the detection of mutations in the coding sequence of 50 genes (Thermo Fisher Scientific); and the Ion Ampliseq 50-Gene Assay for the detection of mutations in the coding sequence of 50 genes (Thermo Fisher Scientific).

In the KURA KO-TIP-001 trial data set, for genomic sequencing, local testing was used and included platforms such as OncoDNA, Foundation One, Memorial Sloan Kettering-Impact, as well as nonspecified local hospital testing platforms.

Statistical Methods for Demographics and Comutation Comparisons

In the FMI cohort, for the mutation and comutation analysis, Fisher's exact tests were used to determine enrichment of gene alterations in *HRAS*-mutant and *HRAS* wild-type samples; clinical data, described above, were analyzed as categorical values using Fisher's exact test. In the MDACC cohort and Kura data set, descriptive statistics were used to describe demographic features listed above (summarized in Appendix Tables A3 and A4).

Definitions of Clinical Outcomes

Disease-free survival (DFS) was defined as the duration of time from the end of primary treatment (surgery or concurrent chemoradiotherapy) for initial locoregional diagnosis to recurrence or metastasis. OS was defined as time from initial primary treatment to death. OS for metastatic

patients (OSmet) was defined as time from treatment for recurrent or metastatic disease to death.

Statistical Methods for Clinical Outcome Analysis

Kaplan-Meier methods were used to estimate median and annual outcomes with 95% CIs. *P* values < .05 were considered statistically significant. All analyses were performed using GraphPad v9.

RESULTS

HRAS Mutation Distribution in HNSCC

First, using four independent data sets, we characterized the mutational landscape of *HRAS*-mutant HNSCC (Table 1) and found that the functional sites of *HRAS*-mutant were strikingly similar across the four data sets (Fig 1). Alterations in codons 12 and 13 accounted for 59% of mutations in the MDACC data set, 70% of the Kura data set and 68% of the FMI data set. In the FMI data set, mutations at codon 13 included two G13_V14 frameshift mutations (Table 1, Appendix Table A1). To validate these findings using an independent publicly available data set, we analyzed 153,834 samples from 137,401 patients available from AACR Project GENIE v.12.[10] database for the prevalence of *HRAS* mutations in HNSCC; mutations in *HRAS* were found in 3.3% of cases (56 of 1,708 cases), and the functional sites of *HRAS*-mutant HNSCC were similar in this data set: Here, alterations in codons 12 and 13 accounted for 64% of functional alterations (n = 36; Table 1, Appendix Table A1). In all cohorts, the most frequent *HRAS* mutation was G12S, which was identified in 25% in MDACC, 30% in Kura, 26% in FMI data set, and 41% of AACR Project GENIE v.12 cohort, respectively.

TABLE 1. Pooled *HRAS* Mutation Distribution: MD Anderson Cancer Center, KURA KO-TIP-001 Trial Participants, Foundation Medicine–Profiled Cases and AACR Project GENIE v.12 Cohort[10]

HRAS Mutation	No. (%) N = 249
G12 C/D/F/N/S/V	98 (39.4)
G13 C/D/E/R/S/V/insG/insGG	69 (28)
Q61 H/K/L/R	60 (24)
A59T	8 (3.2)
D119 H/N	4 (1.6)
Q22K	2 (0.8)
K117	2 (0.8)
F82C	1 (0.4)
A18V	1 (0.4)
V29G	1 (0.4)
D33Y	1 (0.4)
G60D	1 (0.4)
E91Q	1 (0.4)

Values (n) indicate the exact mutation present for each codon, the number of cases for the four cohorts combined and the percentage of occurrence of the *HRAS* mutation.

Aside from mutations in codons 12 and 13, mutations in codon 61 were also frequently present, altered in 31% in the MDACC, 11% in Kura, 27% in FMI data set (n = 42), and 20% of AACR Project GENIE v.12 cohort (n = 11; Table 1, Appendix Table A1). We also analyzed the documented pathogenicity of each *HRAS*-mutant in Table 1, using mutation impact on COSMIC v73,[14] summarized in Appendix Table A2. Using this analysis, only scores ≥ 0.7 are classified as pathogenic; almost all *HRAS* mutations reported in this study were deemed pathogenic (98%, Appendix Table A2).

Clinicopathological Characteristics of *HRAS*-Mutant HNSCC

Next, we examined the clinicopathological features of *HRAS*-mutant HNSCC and identified cases from data sets with available clinical data (combined total of 193 patients [13 from MDACC, 27 from Kura, and 155 from FMI]; there was one overlapping case between the Kura data set and the MDACC data set and a separate single overlapping case between the Kura data set and the FMI data set).

For each data set, we described the patient demographics and tumor characteristics (Appendix Table A4). The median age was similar across the three data sets, median age 55 years (range, 42-74 years) in MDACC, 61 years (range, 48-79 years) in Kura, and 65 years in FMI. Patients were more frequently male (64% in total data set, in 54% in MDACC, 73% in Kura, and 65% in FMI; Appendix Table A4). In all three data sets, *HRAS*-mutant HNSCC occurred with a higher frequency in HPV-negative tumors (78.5%) for cases with available HPV/p16 results: HPV-negative cases in 57% in MDACC, 80% in Kura, and 79% in FMI data set, respectively.

The FMI clinical data set was the largest cohort analyzed, and 155 unique *HRAS*-mutant HNSCC cases were identified from a total of 4,759 HNSCC sequenced, representing a prevalence of 3.3%. Here, we compared clinical demographic features of patients with *HRAS*-mutant versus patients with wild-type HNSCC. There was no significant difference in age or gender distribution: In both *HRAS*-mutant and *HRAS* wild-type groups, most of the patients were 59 years or older (67.7% in *HRAS*-mutant *v* 58.3% in *HRAS* wild-type, Fig 2). Most patients in both *HRAS*-mutant and wild-type were male (65.2% of *HRAS*-mutant *v* 76.3% of *HRAS* wild-type, Fig 2). HPV-positive disease was observed in 20.6% of patients with *HRAS*-mutant HNSCC (32/155), while HPV-positive disease was observed in 32.5% of patients with *HRAS* wild-type (1,485/4,604), Fisher's exact *P* < .0015, which encompasses most patients with HNSCC (Fig 2). Therefore, the majority of *HRAS*-mutant HNSCCs were HPV-negative (78.5%), and *HRAS*-mutant HNSCC appears to enrich for HPV-negative disease. TMB high was less frequent in *HRAS*-mutant (20.7%) compared with *HRAS* wild-type group (27.7%, *P* = .044, Fig 2). Programmed death-ligand 1 data were available for only 13 of
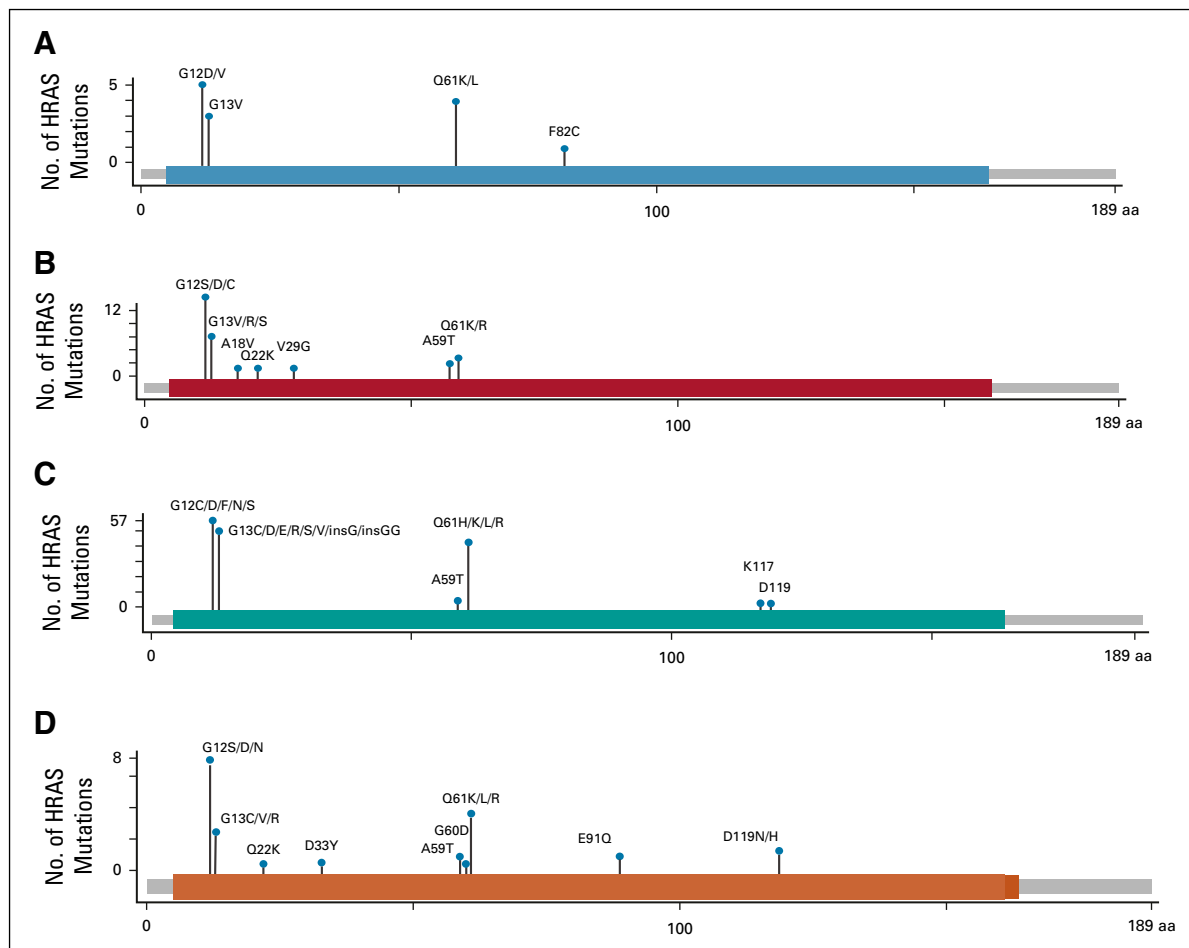
FIG 1. Functional sites of *HRAS* mutations across four data sets: (A) MDACC cohort, (B) Kura cohort, (C) FMI cohort, and (D) AACR GENIE v.12 cohort. Lollipop plots indicate the location of *HRAS* mutations documented in all four cohorts. AACR, American Association for Cancer Research; FMI, Foundation Medicine Inc; Kura, Kura Oncology Inc; MDACC, MD Anderson Cancer Center.

the *HRAS*-mutant group and 305 of the *HRAS* wild-type group (Fig 2).

Comutational Landscape With *HRAS* Mutation in HNSCC

Next, we explored the comutational landscape of *HRAS*-mutant HNSCC. As the FMI data set, with 155 unique *HRAS*-mutant HNSCC, was the largest genomic data set identified in an established platform (324 gene panel and select gene rearrangements), we focused our subsequent analysis and comparisons using this data set. In the FMI data set, in comparison with *HRAS* wild-type HNSCC tumors, mutations in caspase-8 (*CASP8*), telomerase reverse transcriptase (*TERT*), and notch homolog 1, translocation-associated (*NOTCH1*) were the most frequent co-occurring mutations in *HRAS*-mutant HNSCC tumors, observed in 27% (*P* < .00013), 60% (*P* < .00013), and 30.5% of *HRAS*-mutant tumors, respectively (*P* < .0085, Fig 3). Mutations in *CDKN2A* were frequently observed in both *HRAS*-mutant and *HRAS* wild-type patients (53% *v* 41%, *P* < .009), in addition to *TP53* mutations in both groups (50% *v* 64%, *P* < .002; Fig 3). *FGF* amplifications (amplifications in *FGF3*,

FGF4, FGF19) were significantly lower in the *HRAS*-mutant tumors compared with *HRAS* wild-type (4% *v* 19% respectively, *P* < .0003), as well as amplifications in *CCND1* which were present in 4% of *HRAS*-mutant versus 19% of *HRAS* wild-type tumors (*P* < .0001, Fig 3). Notably, in contrast to *KRAS*-mutant non–small-cell lung cancer, *STK11* mutations were a rare phenomenon across all data sets: There were only five patients with *STK11* comutations (3.1%) in the FMI data set and no documented *STK11* comutations in the MDACC or Kura data sets. In addition, there were no *KEAP1* mutations observed in all data sets.

In all data sets, co-occurring mutations in the presence of *HRAS*-mutant were frequently noted: Only 15% (n = 2) of the MDACC data set and 19% (n = 5) of the Kura data set had no documented co-occurring alteration in the presence of *HRAS*-mutant. The most frequent co-occurring mutation across all data sets was *TP53* (MDACC, 46% [n = 6]; Kura, 32% [n = 9]; FMI, 53% [n = 82], Fig 3). In the Kura data set, comutations in *TERT* and *NOTCH1* were also observed, present in 11% and 4% of patients, respectively;
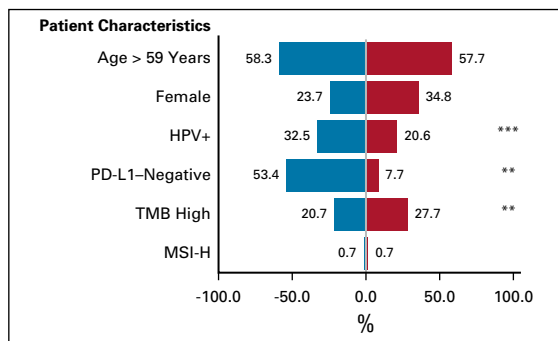
FIG 2. *HRAS*-mutant HNSCC versus *HRAS* wild-type populations in the Foundation Medicine Inc cohort. In this cohort, 155 unique *HRAS*-mutant HNSCC cases were identified from a total of 4,759 HNSCC sequenced. Patients with *HRAS*-mutant HNSCC are denoted in red; patients with *HRAS* wild-type are denoted by blue. Most patients were older than 59 years (n = 105 [67.7% in HRAS-mutant patients, n = 2,660], 58.3% in *HRAS* wild-type); female patients were in the minority in both groups (34.8% of HRAS-mutant patients were female [54/155]; 23.7% of *HRAS* wild-type patients were female [1,081/4,604]). Patients with both *HRAS*-mutant and *HRAS* wild-type HNSCC were more likely to be defined as HPV-negative disease (20.6% [32/155] of patients with *HRAS*-mutant HNSCC; 32.5% [1,485/4,604] of patients with HRAS wild-type, $P < .0015$). $**P < .044$; $***P < .0016$. HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; MSI-H, microsatellite instability High; PD-L1, programmed death-ligand 1; TMB, tumor mutation burden.

PIK3CA was a frequently occurring comutation, present in 26% of patients (n = 7), and there were no documented *CASP8* comutations.

Response to FTase Inhibitor, Tipifarnib, by Mutations in *HRAS*

We next sought to assess whether the location of *HRAS* mutation affected response to tipifarnib therapy, by analyzing the KURA KO-TIP-001 trial participant data set. In this data set, 20 patients with *HRAS*-mutant HNSCC with *HRAS* variant allele frequency ≥ 20% received tipifarnib and had evaluable disease response. Treatment responses were not codon-specific, and documented partial responses were achieved in patients with *HRAS*-mutant HNSCC in codon 12, 13, 61, and 22 (Fig 4): In total, 11 patients achieved partial response as best response (55%). This included seven patients with G12C/D/S mutations, two with G13R/V mutations, one with Q61K, and one with Q22K (Fig 4). Six patients had stable disease as best overall response (30%), and five of six of these patients had documented tumor shrinkage.

Clinical Outcomes and Survival Analyses in *HRAS*-Mutant HNSCC

Complete clinical and outcome data were available for two of four data sets (MDACC and Kura) which were then analyzed. Appendix Table A3 summarizes clinical and pathological characteristics. Most patients had stage IV disease at diagnosis (63% in MDACC data set and 85% in

Kura data set), and primary definitive treatment, while heterogeneous in both groups, typically involved surgery followed by adjuvant or upfront concurrent chemoradiotherapy. In the metastatic setting, chemotherapy (50% in MDACC data set and 37% in Kura data set) as well as immunotherapy were frequently used (38% in MDACC data set and 48% in Kura data set).

In the Kura data set, all patients received tipifarnib as part of a clinical trial (ClinicalTrials.gov identifier: NCT02383927). Among the 27 patients in the Kura data set who had primary definitive HNSCC treatment, most patients (67%, n = 18) progressed within 6 months of definitive treatment (range, 7-48 months). In the Kura data set for patients, DFS was 4.0 months (95% CI, 2.0 to 6.0; range, 1-63 months; n = 27) and OS was 25.5 months (95% CI, 18.0 to 48.0; range, 4-94 months; n = 27; Figs 5A-5C); the median OSmet in the Kura data set was 15.0 months (95% CI, 2.0 to 33.0; range, 1-47 months; n = 27; Fig 5B). In the MDACC data set, we performed the same analyses (one patient received tipifarnib as part of clinical trial, ClinicalTrials.gov identifier: NCT02383927, and was excluded from MDACC analysis but included in Kura analysis). Here, 50% of patients progressed after definitive therapy within 6 months. DFS was 4.0 months (95% CI, 1.0 to 36.0; n = 12) and OS was 15 months (95% CI, 6.0 to 52.0; n = 12; Figs 5A-5C); the median OSmet was 12 months (95% CI, 1.0 to 36.0; n = 12; Fig 5B).

DISCUSSION

In this retrospective, multicohort study of 249 cases of *HRAS*-mutant HNSCC is the largest genomic data set of *HRAS*-mutant HNSCC reported to date. Our study confirms that *HRAS* mutations occur at a rate of 3%-4% in patients with recurrent or metastatic HNSCC,[6] and *HRAS* mutations occur in both HPV-positive and HPV-negative HNSCC, though more frequently in HPV-negative tumors, supporting a more focused testing strategy in HPV-negative patients in resource-constraint settings. We describe the mutation and comutational landscape of *HRAS*-mutant HNSCC: By leveraging four independent data sets, we identify *HRAS* G12S as the most common *HRAS* mutation and confirm responses to tipifarnib are not codon-specific, as responses to tipifarnib were seen across codons, 12, 13, and 61. Thus, we confirm that all functional *HRAS* mutations can benefit from FTIs, such as tipifarnib.

Genomic analysis has revealed a complex mutational profile for *HRAS*-mutant HNSCC.[15,16] In contrast to *HRAS* wild-type HNSCC tumors, we find *CASP8*, *TERT*, and *NOTCH1* as the most frequent co-occurring mutations with *HRAS* mutations.[15,16] *CASP8* is one of the most frequently mutated genes in HNSCC, and *CASP8* mutations are known to be associated with poor survival.[17] Additionally, *CASP8* has been shown to correlate with *HRAS*-mutant in HNSCC and could be permissive for *HRAS*-mutant.[17] We present mutations in *NOTCH1* and *TERT* as novel comutations of
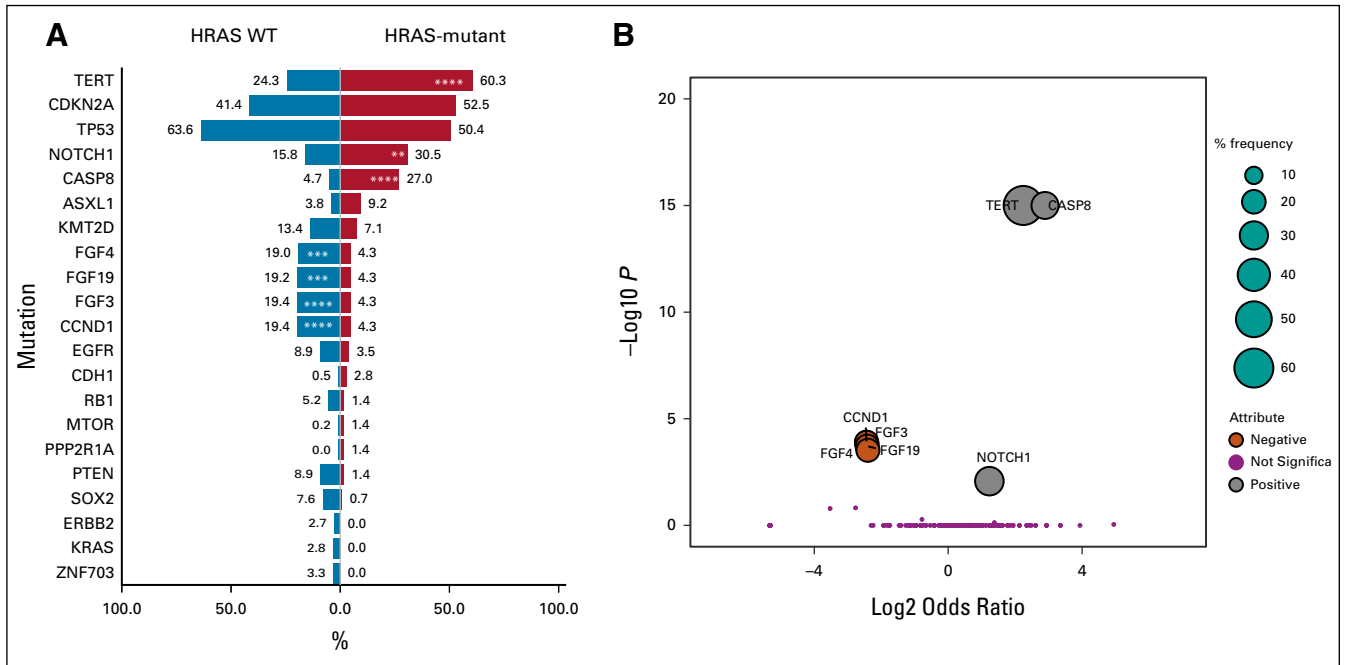
FIG 3. Comutational profile of *HRAS*-mutant versus WT populations in the Foundation Medicine Inc cohort. *P* values were calculated using a Fisher's exact test and were corrected for multiple testing. Data displayed using (A) bidirectional bar chart and (B) volcano plot. **$P <$ .0085; ***$P <$ .0003; ****$P <$ .00013. Mutations in *CASP8*, *NOTCH1*, and *TERT* are identified as statistically significant comutations in the *HRAS*-mutant HNSCC population. Amplifications in *FGF* and *CCND1* are identified as statistically significant and mutually exclusive in the HRAS-mutant HNSCC population. HNSCC, head and neck squamous cell carcinoma; WT, wild-type.

potential significance in *HRAS*-mutant HNSCC. *TERT* has been shown to contribute to cancer development and progression via multiple activities, including its telomere lengthening-dependent and independent functions,[18] and *TERT* promoter mutations are prognostic in many cancers. Genomic sequencing of HNSCC has suggested that *NOTCH1* acts as a tumor suppressor gene, and it has been documented as the second most frequent alteration in HNSCC, with an incidence of 15%-19%.[19,20] Thus, while mutations in *NOTCH1* and *TERT* have been observed in HNSCCs[15,21,22] (TERT in HPV-negative HNSCC), their

respective roles in the setting of *HRAS*-mutant HNSCC as comutations have not been clearly defined.

Amplification of 11q13 is frequently observed in solid tumors including HNSCC, and several genes in this amplicon, such as *FGF3*, *FGF4*, *FGF19*, and *CCND1*, are associated with tumorigenesis.[23,24] Interestingly, we found that this amplicon (*FGF3*, *FGF4*, *FGF19*, *CCND1*) is less likely to occur in *HRAS*-mutant HNSCC tumors compared with *HRAS* wild-type HNSCC. In contrast to *K-RAS*-mutant non–small-cell lung cancer, we found that comutations in

FIG 4. Best overall response to tipifarnib by *HRAS* mutation in the Kura data set. Twenty patients were evaluable in the Kura data set. Tumor responses were demonstrated across codons 12, 13, 61, and 22. *Denotes the three patients with human papillomavirus–positive disease who achieved stable disease as best response. PR, partial response.
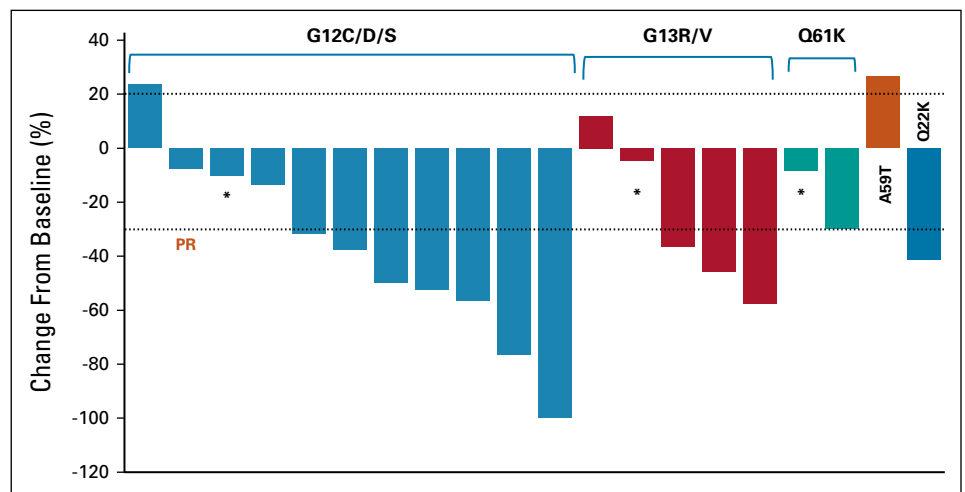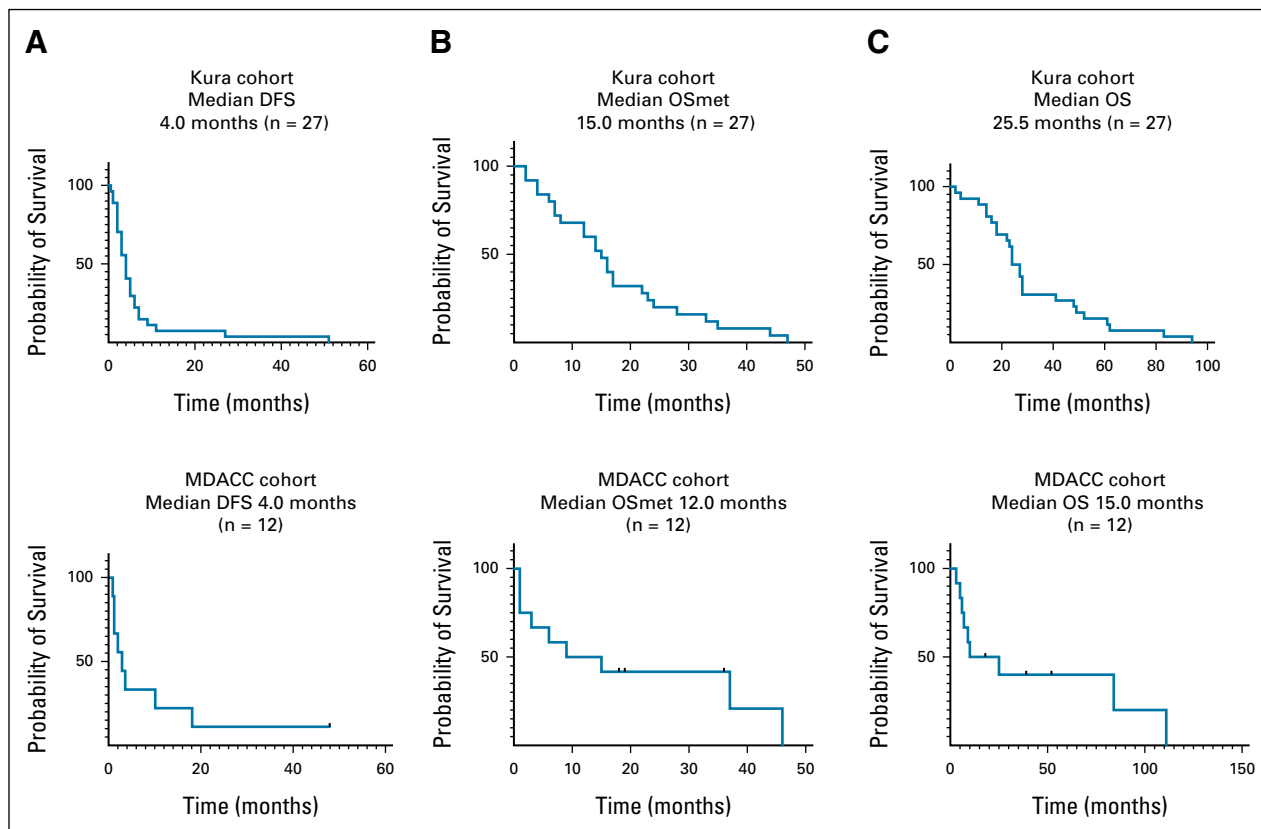
FIG 5. Survival analyses performed in clinical data sets (MD Anderson Cancer Center and Kura): (A) Median DFS, (B) median OSmet, and (C) median OS in each respective cohort. DFS, disease-free survival; Kura, Kura Oncology, Inc; MDACC, OS, overall survival; OSmet, metastatic OS.

KEAP1 were absent and *STK11* mutations were rare (3.1%). Our data suggest that *HRAS*-mutant HNSCC is a distinct biological entity with a unique comutational profile.

Clinically, real-world data in recurrent and/or metastatic HNSCC have overall shown poor outcomes with reported median progression-free survivals of 4.8-7.1 months and OS 7.1-11.7 months.[25,26] Analysis of our MDACC clinical data set confirmed that clinical outcomes in *HRAS*-mutant HNSCC, overall, are poor, with a high rate of relapse following primary definitive treatment and relatively short DFS (4.0 months) and OS (15.0 months). Tipifarnib has demonstrated encouraging efficacy in patients with *HRAS*-mutant HNSCC,[9] and the use of tipifarnib in the Kura data set demonstrated improved OS compared with the MD Anderson cohort. While the DFS were comparable in MDACC (4.0 months) and Kura (4.0 months) data sets, the difference of OS between MDACC (15.0 months) and Kura (25.5 months) can be partially explained as clinical trial patients are typically more robust; it is also highly likely that the addition of tipifarnib to their arsenal of therapeutic options provided benefit, as tipifarnib demonstrated PFS of 5.6 months in the prior report.[27] Altogether, our clinical data highlight the poor prognosis of this molecularly defined HNSCC subtype, while emphasizing the importance of FTIs, such as tipifarnib in this area of unmet need.

Our study has several limitations. While we were able to leverage four independent data sets, including the recent AACR GENIE v.12 data set,[10] two of these cohorts were small, and the largest data sets did not have full clinical outcome information. As this is a rare oncogene-driven subset of HNSCC, our combined data sets nonetheless provide unique insights into this molecularly defined disease. The complete comutation analysis was only performed with one large data set. In this analysis, we are also limited by not functionally validating these mutations using experimental models, which should be a focus of future work.

In conclusion, we present the largest data set of *HRAS*-mutant HNSCC and confirm *HRAS*-mutant HNSCC as a distinct clinical and genomic entity. We demonstrate that FTI tipifarnib is active across *HRAS* codons, and while clinical outcomes in *HRAS*-mutant HNSCC, overall, are poor, our data highlight the importance of FTIs in this rare genomically defined HNSCC subgroup. We confirm that, although more likely to be associated with HPV-negative disease, *HRAS* mutation can occur in both HPV-positive and HPV-negative HNSCC and we observe clinical benefit from FTase inhibition in patients with *HRAS*-mutant HNSCC with both HPV-positive and HPV-negative tumors. Comutations of significance in HNSCC are present in *HRAS*-mutant HNSCC (*NOTCH1*, *CASP8*, *TP53*, *CDKN2A*) and demonstrate *FGF* and *CCND1* amplifications

are mutually exclusive with *HRAS* mutation. Understanding the mutational, genomic landscape of HNSCC has led to the development of better therapeutic approaches in this disease

and our data provide additional insights into this rare genomic subset of HNSCC which may be of interest in future clinical trial design.

AFFILIATIONS

[1]Department of Investigational Cancer Therapeutics, University of Texas MD Anderson Cancer Center, Houston, TX
[2]Department of Thoracic/Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX
[3]Cancer Genomics Research, Foundation Medicine Inc, Cambridge, MA
[4]Clinical Development, Kura Oncology Inc, Boston, MA
[5]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX

CORRESPONDING AUTHOR

Xiuning Le, MD, PhD, Department of Thoracic/Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030; e-mail: xle1@mdanderson.org.

AUTHOR CONTRIBUTIONS

Conception and design: Niamh Coleman, Jeffrey Myers, John Heymach, Maura Gillison, Xiuning Le
Administrative support: John Heymach, Maura Gillison
Provision of study materials or patients: Edward Park, David Hong
Collection and assembly of data: Niamh Coleman, Kathrina L. Marcelo, Edward Park, Binaifer Balsara, Lee A. Albacker, David Hong, Xiuning Le
Data analysis and interpretation: Niamh Coleman, Kathrina L. Marcelo, Julia F. Hopkins, Nusrat Israr Khan, Robyn Du, Lingzhi Hong, Mollie Leoni, Curtis Pickering, Lee A. Albacker, Maura Gillison, Xiuning Le
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Kathrina L. Marcelo
Employment: Caris Life Sciences
Stock and Other Ownership Interests: Caris Life Sciences
Travel, Accommodations, Expenses: Caris Life Sciences

Julia F. Hopkins
Employment: Foundation Medicine
Stock and Other Ownership Interests: Roche

Edward Park
Employment: Kura Oncology, Arvinas
Stock and Other Ownership Interests: Kura Oncology, Arvinas

Binaifer Balsara
Employment: Kura Oncology
Stock and Other Ownership Interests: Kura Oncology

Mollie Leoni
Employment: Kura Oncology, Kyowa Kirin International
Stock and Other Ownership Interests: Kura Oncology
Travel, Accommodations, Expenses: Kura Oncology, Kyowa Kirin International

Curtis Pickering
Patents, Royalties, Other Intellectual Property: I receive small royalties each year from a biomarker patent that was developed while I was at the University of California San Francisco. I am not aware of who is paying the royalties to the University of California, and I just receive a check each year from the university. That biomarker is unrelated to anything I am doing now

John Heymach
Stock and Other Ownership Interests: Cardinal Spine, Bio-Tree
Consulting or Advisory Role: AstraZeneca, Bristol Myers Squibb, Spectrum Pharmaceuticals, Guardant Health, Hengrui Pharmaceutical, GlaxoSmithKline, EMD Serono, Takeda, Sanofi/Aventis, Genentech/Roche, Boehringer Ingelheim, Mirati Therapeutics, Janssen, Nexus Health Systems, Pneuma Respiratory, Lilly (Inst)
Speakers' Bureau: IDEOlogy Health, MJH Life Sciences
Research Funding: AstraZeneca (Inst), Spectrum Pharmaceuticals, GlaxoSmithKline
Patents, Royalties, Other Intellectual Property: Licensing agreement between Spectrum and MD Anderson (including myself) regarding intellectual property for treatment of EGFR and HER2 exon 20 mutations

Lee A. Albacker
Employment: Foundation Medicine
Stock and Other Ownership Interests: Roche

David Hong
Stock and Other Ownership Interests: OncoResponse, Telperian, MolecularMatch
Consulting or Advisory Role: Bayer, Guidepoint Global, Gerson Lehrman Group, Alphasights, Axiom Biotechnologies, Medscape, Numab, Pfizer, Takeda, Trieza Therapeutics, WebMD, Infinity Pharmaceuticals, Amgen, Adaptimmune, Boxer Capital, EcoR1 Capital, Tavistock Life Sciences, Baxter, COG, Genentech, GroupH, Janssen, Acuta, HCW Precision, Prime Oncology, ST Cube, Alkermes, AUM Biosciences, Bridgebio, Cor2Ed, Gilead Sciences, Immunogen, Liberum, Oncologia Brasil, Pharma Intelligence, Precision Oncology Experimental Therapeutics, Turning Point Therapeutics, ZIOPHARM Oncology, Cowen, Gennao Bio, MedaCorp, YingLing Pharma, RAIN
Research Funding: Genentech (Inst), Amgen (Inst), Daiichi Sankyo (Inst), Adaptimmune (Inst), AbbVie (Inst), Bayer (Inst), Infinity Pharmaceuticals (Inst), Kite, a Gilead Company (Inst), MedImmune (Inst), National Cancer Institute (Inst), Fate Therapeutics (Inst), Pfizer (Inst), Novartis (Inst), Numab (Inst), Turning Point Therapeutics (Inst), Kyowa (Inst), Loxo (Inst), Merck (Inst), Eisai (Inst), Genmab (Inst), Mirati Therapeutics (Inst), Mologen (Inst), Takeda (Inst), AstraZeneca (Inst), Navire (Inst), VM Pharma (Inst), Erasca Inc (Inst), Bristol Myers Squibb (Inst), Adlai Nortye (Inst), Seattle Genetics (Inst), Deciphera (Inst), Pyramid Biosciences (Inst), Lilly (Inst), Endeavor BioMedicines (Inst), F. Hoffmann LaRoche (Inst), Ignyta (Inst), Teckro (Inst), TCR2 Therapeutics (Inst)
Travel, Accommodations, Expenses: Genmab, Society for Immunotherapy of Cancer, Bayer Schering Pharma, ASCO, AACR, Telperian

Maura Gillison
Consulting or Advisory Role: Bristol Myers Squibb, Merck, EMD Serono, BioNTech, Shattuck Labs, Bayer, Debiopharm Group, Ipsen, Gilead Sciences, Bicara Therapeutics, BioNTech, Nektar, Istari, LLX Solutions, OncLive, Seattle Genetics, Kura Oncology, Mirati Therapeutics, Sensei Biotherapeutics, Eisai, Exelixis, ITeos Therapeutics, Caladrius Biosciences
Research Funding: Bristol Myers Squibb (Inst), Genocea Biosciences (Inst), Cullinan Oncology (Inst), Genentech (Inst), Agenus (Inst), Kura Oncology (Inst)

Xiuning Le

REFERENCES

1. Sklan A, Collingridge D: Treating head and neck cancer: For better or for worse? Lancet Oncol 18:570-571, 2017

2. Burtness B, Harrington KJ, Greil R, et al: Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): A randomised, open-label, phase 3 study. Lancet 394:1915-1928, 2019

3. Cox AD, Der CJ: Ras history: The saga continues. Small GTPases 1:2-27, 2010

4. Hong DS, Fakih MG, Strickler JH, et al: KRAS G12C inhibition with sotorasib in advanced solid tumors. N Engl J Med 383:1207-1217, 2020

5. Li S, Balmain A, Counter CM: A model for RAS mutation patterns in cancers: Finding the sweet spot. Nat Rev Cancer 18:767-777, 2018

6. Mountzios G, Rampias T, Psyrri A: The mutational spectrum of squamous-cell carcinoma of the head and neck: Targetable genetic events and clinical impact. Ann Oncol 25:1889-1900, 2014

7. Untch BR, Dos Anjos V, Garcia-Rendueles MER, et al: Tipifarnib Inhibits HRAS-Driven dedifferentiated thyroid cancers. Cancer Res 78:4642-4657, 2018

8. End DW, Smets G, Todd AV, et al: Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. Cancer Res 61:131-137, 2001

9. Ho AL, Brana I, Haddad R, et al: Tipifarnib in head and neck squamous cell carcinoma with HRAS mutations. J Clin Oncol 39:1856-1864, 2021

10. André F, Arnedos M, Baras AS, et al: AACR project genie: Powering precision medicine through an international consortium. Cancer Discov 7:818-831, 2017

11. Frampton GM, Fichtenholtz A, Otto GA, et al: Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 31:1023-1031, 2013

12. Chalmers ZR, Connelly CF, Fabrizio D, et al: Analysis of 100, 000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9:34, 2017

13. Luthra R, Patel KP, Routbort MJ, et al: A targeted high-throughput next-generation sequencing panel for clinical screening of mutations, gene amplifications, and fusions in solid tumors. J Mol Diagn 19:255-264, 2017

14. Shihab HA, Rogers MF, Gough J, et al: An integrative approach to predicting the functional effects of non-coding and coding sequence variation. Bioinformatics 31:1536-1543, 2015

15. Pickering CR, Zhang J, Yoo SY, et al: Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. Cancer Discov 3:770-781, 2013

16. Farah CS: Molecular landscape of head and neck cancer and implications for therapy. Ann Transl Med 9:915, 2021

17. Uzunparmak B, Gao M, Lindemann A, et al: Caspase-8 loss radiosensitizes head and neck squamous cell carcinoma to SMAC mimetic–induced necroptosis. JCI Insight 5:139837, 2020

18. Yuan X, Larsson C, Xu D: Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: Old actors and new players. Oncogene 38:6172-6183, 2019

19. Agrawal N, Frederick MJ, Pickering CR, et al: Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 333:1154-1157, 2011

20. Stransky N, Egloff AM, Tward AD, et al: The mutational landscape of head and neck squamous cell carcinoma. Science 333:1157-1160, 2011

21. Moreira A, Poulet A, Masliah-Planchon J, et al: Prognostic value of tumor mutational burden in patients with oral cavity squamous cell carcinoma treated with upfront surgery. ESMO Open 6:100178, 2021

22. Kim H, Kwon MJ, Park B, et al: Negative prognostic implication of TERT promoter mutations in human papillomavirus–negative tonsillar squamous cell carcinoma under the new 8th AJCC staging system. Indian J Surg Oncol 12:134-143, 2021 (suppl 1)

23. Tsui IFL, Poh CF, Garnis C, et al: Multiple pathways in the FGF signaling network are frequently deregulated by gene amplification in oral dysplasias. Int J Cancer 125:2219-2228, 2009

24. Chen C, Zhang Y, Loomis MM, et al: Genome-wide loss of heterozygosity and DNA copy number aberration in HPVNegative oral squamous cell carcinoma and their associations with disease-specific survival. PLoS One 10:e0135074, 2015

25. Pontes F, Garcia AR, Domingues I, et al: Survival predictors and outcomes of patients with recurrent and/or metastatic head and neck cancer treated with chemotherapy plus cetuximab as first-line therapy: A real-world retrospective study. Cancer Treat Res Commun 27:100375, 2021

26. Depenni R, Cossu Rocca M, Ferrari D, et al: Clinical outcomes and prognostic factors in recurrent and/or metastatic head and neck cancer patients treated with chemotherapy plus cetuximab as first-line therapy in a real-world setting. Eur J Cancer 115:4-12, 2019

27. Ho AL, Hanna GJ, Scholz CR, et al: Preliminary activity of tipifarnib in tumors of the head and neck, salivary gland and urothelial tract with HRAS mutations. J Clin Oncol 38, 2020 (15 suppl; asbtr 6504)

■ ■ ■

APPENDIX 1. SUPPLEMENTARY MATERIAL AND RESULTS

Supplementary Methods

Detail of each of the four data sets.

MD Anderson Cancer Center cohort. The study was approved by the Institutional Review Boards at MD Anderson Cancer (January 2004 to June 2020, per protocol LAB02-039 and PA14-0947). Data were pooled from an institutional data set with complete clinical information including age, sex, primary cancer site, smoking status, human papillomavirus (HPV) status, treatments received, date of diagnosis, date of death, treatment dates, and genomic information for each patient (*HRAS* mutation status and comutational data, testing platform varies).

Kura cohort. For the cases used from the KURA KO-TIP-001 study (ClinicalTrials.gov identifier: NCT02383927), approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817, September 2015 to April 2020). These studies were conducted in accordance with ethical guidelines including the Declaration of Helsinki and US Common Rule. Consented data that can be released are included in the article and its supplementary files. Clinical data were collected for each patient, including age, sex, race, smoking status, HPV status (if known), primary cancer, primary definitive treatment received, metastatic treatments, date of diagnosis, date of death, treatment dates, and genomic information.

Foundation Medicine cohort. Patient samples were collected from 2013 to 2020. Full genomic data for patients with HRAS-mutant head and neck squamous cell carcinoma and HRAS wild-type head and neck squamous cell carcinoma were available for analysis, including HRAS mutation and comutational data. Clinical data regarding treatment, date of diagnosis, and treatment outcomes were not available for this cohort. Clinical data that were available for patients in this cohort included age, sex, HPV status, TMB high/low status, microsatellite stability, and programmed death-ligand 1 (PD-L1) status.

AACR GENIE v.12 cohort. AACR Project GENIE is a publicly accessible cancer registry of real-world data assembled through data sharing between 19 leading international cancer centers. The data set was analyzed in July 2022, to access *HRAS* mutation distribution data. No clinical data were available in this cohort.

Histology. PD-L1 status was determined through immunohistochemistry performed on formalin-fixed paraffin-embedded tissue sections with 22C3 (Dako/Agilent, Santa Clara, CA). A board-certified pathologist determined the tumor proportion score (TPS) for each sample as defined by the assay package insert for use as a companion diagnostic. The TPS is the proportion of tumor cells exhibiting linear membranous staining out of all tumor cells and is reported as a percentage (0%-100%). PD-L1 expression was summarized as negative ($< 1\%$ TPS) or positive ($\geq 1\%$ TPS). The pathology laboratory established performance characteristics for this assay per the requirements of the Clinical Laboratory Improvement Amendments '88 and in accordance with College of American Pathologists checklist requirements and guidance.

10

TABLE A1. *HRAS* Mutation Distribution in Each Cohort, MDACC (2004-2020), KURA KO-TIP-001 Trial Participants, FMI-Profiled Cases, and AACR Project GENIE v.12 Cohort

MDACC (n = 12)	Kura (n = 27)	FMI (n = 154)	AACR GENIE v.12 (n = 56)
G12S/V/D: 42% (n = 5)	G12S/V/D: 44% (n = 12)	G12 C/D/F/N/S: 36% (n = 56)	G12S/D/N: 40% (n = 25)
G13V: 17% (n = 2)	G13V/R/S: 26% (n = 7)	G13 C/D/E/R/S/V/insG/ insGG: 32% (n = 49)	G13R/C/V/D: 17.5% (n = 11)
	A18V: 4% (n = 1)		
	Q22K: 4% (n = 1)		Q22K: 1.6% (n = 1)
	V29G: 4% (n = 1)		D33Y: 1.6% (n = 1)
	A59T: 7% (n = 2)	A59T: 2.6% (n = 4)	A59T: 3%. (n = 2)
Q61K/L: 33% (n = 4)	Q61K/R: 11% (n = 3)	Q61 H/K/L/R: 27% (n = 42)	G60D: 1.6% (n = 1)
			Q61K/L/H/R: 17.5% (n = 11)
F82C: 8% (n = 1)			E91Q: 1.6% (n = 1)
		K117: 1.3% (n = 2)	
		D119: 0.7% (n = 1)	D119H/N: 5% (n = 3)

NOTE. Values (n) indicate the exact mutation present for each codon, the number of cases for the overall four cohorts.

Abbreviations: AACR, American Association for Cancer Research; FMI, Foundation Medicine Inc; Kura, Kura Oncology Inc; MDACC, MD Anderson Cancer Center.

TABLE A2. Summary of the Pathogenic Score of Each HRAS Mutation Identified Using Mutation Impact on COSMIC v73

HRAS Mutation	Pathogenic Score
G12C	0.99
G12D	0.99
G12N	0.99
G12V	0.99
G12S	0.99
G13C	0.99
G13V	0.99
G13D	0.99
G13R	0.99
G13S	0.99
A18V	0.99
Q22K	1.00
A59T	0.97
Q61K	0.99
Q61L	0.98
Q61R	0.97
K117	0.99
D119H	0.97
D119N	0.97
D33Y	0.99
E91Q	0.99
G12F	NA
G13E	NA
F82C	NA
V29G	NA
Q61H	NA
G13insG/GG	NA

NOTE. These scores have been derived from the new FATHMM-MKL algorithm, which predict the functional, molecular, and phenotypic consequences of protein missense variants using hidden Markov models. To highlight the most significant data in COSMIC, only scores ≥ 0.7 are classified as pathogenic.

Abbreviation: NA, no results available.

TABLE A3. Clinical and Pathological Characteristics of the MDACC and Kura Cohorts

Data Set	MDACC (n = 11)	Kura (n = 27)
Primary site of disease, No. (%)		
Oral cavity	7 (64)	16 (59)
Base of tongue	3 (27)	3 (11)
Tonsil/tonsillar fossa	1 (9)	1 (4)
Other	0	7 (26)
Initial stage at diagnosis, No. (%)		
I	1 (9)	0
II	0	2 (7)
III	3 (27)	2 (7)
IVa	5 (45)	23 (85)
IVb	2 (18)	
Primary definitive treatment, No. (%)		
Surgery => XRT with or without chemo	5 (45)	14 (52)
Concurrent chemo-XRT	2 (18)	1 (4)
Induction => definitive therapy	2 (18)	5 (19)
Surgery => XRT => salvage chemo/XRT	0	4 (15)
Surgery alone	1 (9)	2 (7)
XRT alone	1 (9)	1 (4)
Exposure to platinum in metastatic setting, No. (%)	4 (50)	10 (37)
Exposure to IO in metastatic setting, No. (%)	3 (38)	13 (48)

Abbreviations: IO, immunotherapy; Kura, Kura Oncology, Inc; MDACC, MD Anderson Cancer Center; XRT, radiotherapy.

TABLE A4. Patient Demographics of *HRAS*-Mutant Head and Neck Squamous Cell Carcinoma in the Three Cohorts: MDACC (2004-2020), KURA KO-TIP-001 Trial Participants, and FMI-Profiled Cases

Data Set	MDACC (n = 13)	Kura (n = 27)	FMI (n = 155)
Median age, years	55	61	65
Sex, % cases			
Male	54	73	65
Female	46	27	35
HPV status, No. (%)			
Positive	3 (43)	3 (20)	38 (21)
Negative	4 (57)	12 (80)	139 (79)

NOTE. HPV cases include only cases which were tested.

Abbreviations: FMI, Foundation Medicine Inc; HPV, human papillomavirus; Kura, Kura Oncology, Inc; MDACC, MD Anderson Cancer Center.