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Efficacy of axitinib in metastatic head and neck cancer using novel radiographic response criteria

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Axitinib in head and neck cancer

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Conflict of Interest Statement

P. L. S. and J. C. B. have applied for provisional patents* (not awarded yet) for companion diagnostics related to the data presented in this publication. The remainder of the authors declare no potential conflicts of interest.

Author Contributions Statement

Paul Swiecicki: Conceptualization, data curation, formal analysis, methodology, resources, manuscript writing, and editing. **Emily Bellile**: Data curation, formal analysis, methodology, manuscript writing, and editing. **Collin Brummel**: Data curation, formal analysis, and manuscript editing. **J Chad Brenner**: Formal analysis, methodology, manuscript writing, and editing. **Francis Worden**: Conceptualization, resources, and manuscript editing.

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Lay Summary

Metastatic head and neck squamous cancer is an incurable disease which has limited treatment options and a poor prognosis. In this study, we are the first to demonstrate the targeted oral drug axitinib improves survival in patients with heavily pre-treated metastatic head and neck cancer. Furthermore, we observed that patients whose tumors had specific mutations derived the greatest benefit from from therapy. Investigation of axitinib in a genomic biomarker selected population alone or in combination with immunotherapy is warranted.

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This manuscript demonstrates that treatment with axitinib improves survival in patients with heavily pre-treated R/M HNSCC, alternate response criteria enables identification of patients with atypical radiographic responses, and patients with PI3K pathway alterations may derive exceptional benefit from therapy. Clinically, this study provides evidence for evaluation of axitinib in a genomic biomarker selected population alone or in combination with immunotherapy.

Abstract

Background: There are limited treatment options in unresectable recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC). Vascular endothelial growth factor is of significant interest for targeted therapy in R/M HNSCC given its central role in tumorigenesis and immune suppression. Axitinib is a potent inhibitor of VEGFR1-3, PDGFR, and c-kit and offers such an approach.

Methods: We report the results of a phase II trial evaluating axitinib in R/M HNSCC using the Choi Criteria for radiographic response assessment. The primary endpoint of this trial was 6 month overall survival.

Results: Twenty-nine patients were enrolled and 28 were evaluable for response. Patients were heavily pre-treated with 61% having had at least one previous systemic treatment in the metastatic setting (range 0-5). The median overall survival of 9.8 months with a 6 month overall survival was 70% which met the protocol defined criteria for clinical efficacy. Best overall response rate was 42%. Correlative analyses demonstrated that PI3K signaling pathway alterations were associated with an increased response to therapy (75% versus 17%). A marked response to therapy was seen in a subgroup of patients who were treated with an immune checkpoint inhibitor after progression on axitinib.

Conclusions: Treatment with axitinib is associated with improved survival in patients with heavily pre-treated head and neck cancer and PI3K pathway alterations may serve as a biomarker for response. Further investigation is warranted to evaluate axitinib in biomarker selected populations, especially in combination with immune checkpoint inhibitor therapy.

Keywords:

Head and neck cancer, axitinib, vascular endothelial growth factor receptor inhibitor, PI3K, Choi Criteria

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Main Text

Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is the 6th most common cancer with 600,000 new cases worldwide each year with an incidence rate that is increasing at an unprecedented rate due to the high prevalence of human papilloma virus (HPV)-induced HNSCC[1]. In fact, oropharyngeal cancer is one of only four cancers increasing in incidence in the United States[2]. Although the majority of patients with HNSCC are cured with multimodality therapy, a significant proportion develop unresectable recurrent or metastatic HNSCC (R/M HNSCC). Despite the recent development of programmed death-1(PD-1) inhibitors, response rates remain low due to variability within the immune micro-environment[3]. Even with these novel therapies, the median survival for patients newly diagnosed with R/M HNSCC is approximately 12 months[4].

With increasing molecular characterization of head and neck squamous cell carcinoma, there has been significant interest in targeted therapy[5]. Vascular endothelial growth factor (VEGF) dysregulation has been identified as a crucial process in R/M HNSCC in not only angiogenesis, but also progression, immunosuppression, and immune tolerance[6, 7]. Furthermore, VEGF overexpression is associated with advanced disease and poor prognosis[8, 9]. Given this central role in advanced disease and tumorigenesis, VEGF inhibition is of significant interest as a candidate for targeted therapy.

Axitinib is a multi-receptor tyrosine kinase approved in renal cell carcinoma which inhibits several isoforms of VEGF receptor (VEGFR 1, 2, and 3). Furthermore, it has inhibitory activity against PDGFR and downstream effectors of EGFR both of which are commonly disrupted and contribute to head and neck tumorigenesis[5, 10, 11]. Given this mechanism of action and known molecular alterations in R/M HNSCC, it seems to be a promising agent for clinical assessment.

We previously reported a phase II study evaluating axitinib in patients with heavily pretreated R/M HNSCC. This work demonstrated a low response (7%) rate with single agent axitinib, however a significant proportion of patients had stable disease (70%) with radiographic findings consistent with treatment response[12]. Moreover, the population had an impressive overall survival (10.9 months) suggesting that efficacy was perhaps was not captured. Hence we postulated that axitinib held significant anti-tumor activity in R/M HNSCC but RECIST criteria failed to appropriately capture responders and may inappropriately suggested tumor progression. Differential manifestations of response have been seen with the use of tyrosine kinase inhibitors (ie swelling, cystic attenuation) which have the potential of abhorrently being interpreted as progressive disease by RECIST prompting the development of the Choi Criteria[13].

Based on these findings, we initiated a new follow up phase II study to investigate the clinical activity of axitinib in R/M HNSCC using the Choi Criteria for response assessment. Our hypothesis was that axitinib would have significant anti-tumor activity as judged by the Choi Criteria and result in an improvement in the 6 month overall survival compared to a historical control.

Materials and Methods

Patient eligibility

This was a phase 2 open label trial approved by the Institutional Review Board (IRBMED) of the University of Michigan Rogel Cancer Center (NCT02762513). All patients provided written informed consent. Patients ≥ 18 years old with histologically documented unresectable recurrent or metastatic head and neck squamous cell carcinoma were eligible. All patients were required to have the presence of measurable disease by CT scan or cutaneous lesions ≥ 10 mm not assessable on imaging but present on physical exam, ECOG performance status of 0-2, and life expectancy of ≥ 12 weeks. Adequate hematopoietic, hepatic, and renal function were required and defined as: absolute neutrophil count ≥ 1.5×10^9 cell/ml, platelets ≥75,000 cells/mm^3, hemoglobin ≥ 9.0 g/dL, concentrations of total serum bilirubin within 1.5x the upper limit of normal (ULN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5x institutional upper limits of normal unless there were liver metastases in which case AST and ALT within 5.0 x ULN, serum creatinine clearance ≥ 30 ml/min, urinary protein < 2+). Women of childbearing potential must have had a negative serum or urine pregnancy test within 3 days prior to treatment.

Patients with tumors encasing major blood vessels, active hemoptysis (> ½ teaspoon of bright red blood per day), or currently using therapeutic anticoagulation were excluded as were those with gastrointestinal abnormalities resulting in impaired absorption. Treatment with epidermal growth factor receptor inhibitors within 30 days preceding study entrance was prohibited. Patients were excluded if they had uncontrolled hypertension prior to enrollment which was defined as a systolic blood pressure readings >140 mm Hg and/or a diastolic blood pressure readings > 90 mm Hg.

Treatment plan

Enrolled patients underwent a complete history and physical examination, baseline laboratory studies (CBC with differential, comprehensive metabolic profile, TSH, urinalysis) and radiographic staging studies (CT Neck/Chest and others as clinically warranted). If cutaneous lesions were not assessable for response by imaging, pictures of the target lesion(s) were obtained as well. All screening assessments were completed within 28 days prior to the start of treatment.

Patients were initiated on axitinib 5 mg twice daily with a cycle length of 28 days. Dose escalation was planned at 2 weeks (to 7 mg twice daily) and 3 weeks to goal of 10 mg twice daily in the absence of grade 2 or greater toxicities. Patients were seen for toxicity assessment and laboratory assessments (CBC, CMP, TSH, UA) at two weeks, four weeks, and then monthly after treatment initiation. Response to therapy. Dose escalation could be resumed at the next visit if toxicities diminished to grade 1 or less. Treatment was continued until disease progression, unacceptable toxicity, patient withdrawal of consent, or investigator discretion.

Evaluation of response

Response assessment was performed after two cycles of axitinib treatment and continued every two cycles. Radiographic assessments obtained at enrollment were obtained at each time point. Similarly, if physical exam was being used for response assessment of cutaneous lesions, pictures were taken at each time point. Photographs as well as imaging studies were submitted to the University of Michigan Tumor Response and Assessment Core. Radiologic response was determined according to the Choi Criteria[13].

Statistical considerations

Twenty nine patients were enrolled between 8/30/2016 and 10/23/19. The median follow up duration among the study participants was 18 months (range: 1-36) and no patients remain on therapy. Follow-up on patients still alive ranges from 5 to 32 months. Based on our previous study supporting an improvement in survival in patients with R/M HNSCC treated with Axitinib, we designed this expansion study. Although consideration was given to adjusting this original study to a Bayesian expansion trial design, it was ultimately decided to begin a new cohort to test for an improvement in survival under the same assumptions of mortality rate as the previous study. Of note, treatment continuation decisions for this trial were based on Choi criteria that, as previously reported[12], considerably differed from RECIST decisions when evaluated in the original trial.

The primary aim was to compare 6-month overall survival after treatment with Axitinib in patients with unresectable, recurrent or metastatic head and neck cancer to historical rates. Based on results in the literature, we assumed a 6-month mortality rate of 50% under current standard care in this patient population[14]. A sample size of 37 patients was planned to test whether survival after treatment with Axitinib is improved to 70% at 6-months compared to 50% with an upper tailed test of binomial proportion. No interim analyses for activity were planned. Based on observed clinical benefit and slowed accrual rate, an unplanned interim analysis was performed after enrollment of 29 patients. Data was analyzed by the study statistician; a statistically meaningful improvement in survival was identified in this analysis and the decision was made to close to further accrual.

Overall survival (OS) was defined as the time from study enrollment to death from any cause. Six month overall survival was the proportion of patients who received at least one cycle of Axitinib alive 6 months after study enrollment and 95% confidence intervals were estimated using the Wilson score interval method. Treatment-related adverse events were graded according to the Common Terminology for Adverse Events version 4.03. Response rate was defined as the sum of patients with complete response (CR) and partial response (PR) per the Choi Criteria. Statistical analysis was performed with SAS v14.3 software (Carey, NC). Planned correlative analyses included genomic analysis of patients where next generation sequencing results were evaluable.

Results

Patient characteristics

Twenty nine patients were enrolled, one of which died prior to treatment with axitinib. All twenty eight patients who received at least one dose of axitinib were included for toxicity analysis of which the baseline characteristics are summarized in Table 1. The mean age was 63.9 years old (range: 37-80) and the majority of patients (61%, n=17) had an ECOG performance score of 1 indicating mild impairment. The primary site of disease for most patients was the oropharynx (42.6%, n= 13) and the majority of study participants were HPV negative (60.7%, n=17). The majority of patients (61%, n=17) had at least one previous systemic treatment in the metastatic setting with the number or previous lines of treatments ranging from 0-5. Seventeen patients (61%) were refractory to platinum therapy defined as progression within 180 days of chemotherapy and twelve patients (43%) were previously treated with a PD-1 inhibitor.

Toxicity

The median duration of treatment was 3 cycles (range: 1-9).The most common toxicities included fatigue (75%), hypertension (54%), nausea (32%) and diarrhea (25%) (Table 2). Bleeding was observed in 5 patients, including one patient with a grade 3 lower GI bleed, all of which spontaneously resolved and did recur with re-initiation of axitinib. Grade 3 or 4 severe toxicities were seen in 16 patients (57%). Severe toxicities included fatigue (21%), hypertension (7%), and mucositis (7%). No grade 5 events were reported. Overall, observe toxicities were consistent with that previously reported in the literature[15, 16].

Efficacy

The 6 month overall survival was 71% (95% CI: 53-85%) (Table 3). This met the protocol defined criteria for supporting evidence of clinical benefit. The median progression free survival was 3.5 months (95% CI: 2.4-5.4 months) and median overall survival was 9.8 months (95% CI: 5.9-12.2 months) (Figure 1).

Three patients completed trial participation prior to response imaging; one due to adverse effects but was clinically noted to have progressive disease, one of whom died due to progressive disease, and a third whom withdrew from the study. The overall response rate was 42% and a disease control rate of 53%. The waterfall plot in Figure 2A graphically demonstrates the depth of response amongst participants evaluable for response. One patient had a durable complete response. Only one patient with cutaneous squamous cell carcinoma demonstrated a response to therapy. This patient had a mutation in KDR (VEGFR2) and achieved a durable complete response. All of the remaining six patients with cutaneous squamous cell carcinoma had progressive disease.

Given the immunomodulatory potential of VEGFR inhibition we evaluated the treatment response in patients who received PD-1 inhibition as part of their treatment course. Eleven patients were treated with a PD-1 inhibitor prior to treatment with axitinib. Three patients had primary resistance to checkpoint inhibitor therapy, none of whom responded to axitinib (0/3). Eight had acquired resistance to checkpoint inhibitor therapy of which 3 patients had a partial response with axitinib (3/8, 37%), 2 had stable disease (2/8, 25%), and 3 had progressive disease (3/8, 37%). Eleven patients were treated with a PD-1 inhibitor post progression on axitinib with an observed response rate of 45% (5/11). Response assessment demonstrated complete response in one patient (1/11, 9%), partial response in four patients (4/11, 36%), stable disease in one patient (1/11, 9%), and progressive disease was seen in the remaining five (5/11, 45%).

<u>Correlative studies</u>

To evaluate the association between genomic alterations, tumor characteristics, and clinical outcomes we analyzed results from patients who had commercial next generation sequencing previously performed (n=20). The investigators defined a set of genes (sequenced as part of all NGS panels) and recurrent alterations are shown (Figure 3). Importantly, while no mutations were identified in FLT1 (VEGFR1), FLT4 (VEGFR3), PDGFR or KIT, two patients had mutations in KDR (VEGFR2) including a S1100F mutation as well as a patient with two mutant alleles R1032Q and G638R (Supplemental Table 1). The ability of axitinib to inhibit these mutant forms of KDR is unknown; however, the patient with the S110F mutation had a complete response whilst the other had progressive disease. Importantly, 55% (11/20) of the patients had TP53 alterations, 40% (8/20) of the patients harbored alterations to genes in the PI3K pathway, including PTEN and PIK3CA, and, 30% (6/20) of the patients had mutations in either KMT2C (MLL2) or KMT2D (MLL3).

The degree of response and pathway alterations were correlated for exploratory analysis (Figure 2B). The relative response rate for patients with mutations in the PI3K pathway was 75% vs 39% in those which were wild-type (6/8 versus 2/12 patients). In terms of the KMT2C/D pathway, the response rate was 33% in the mutant population versus 50% in the rest of the population (2/6 versus 6/12 patients). Given the differential responses seen between patients with cutaneous squamous cell carcinoma versus non-cutaneous primaries, the response rates were further explored (Table 3). Although sample sizes were limited, mutations in the PI3K pathway were associated with a higher response rate than the wild type population in the non-cutaneous squamous cell carcinoma (86% versus 12%).

Discussion

In this phase 2 study of patients with heavily pretreated unresectable recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC), axitinib demonstrated an improvement in 6 month overall survival compared to a historical controls (70% vs 50%). Furthermore, treatment resulted in significant response rates and lower rates of severe toxicities.

There is increasing recognition of variable radiographic manifestations of response with the advent of novel classes of therapeutics. Most recognized is the 'pseudoprogression' observed with immunotherapy which prompted development of iRECIST to capture atypical responses[17]. The Choi Response Criteria have been best evaluated in gastrointestinal stromal tumors (GISTs) where, compared to RECIST, they have been demonstrated to better predict survival[13]. In our previous trial using RECIST we observed a low RECIST assessed objective response rate, but a paradoxically high impressive overall survival in heavily pre-treated patients[12]. As such, we hypothesized that we were underappreciating treatment responses with the use of RECIST and the Choi Criteria may be more appropriate for discerning patients deriving benefiting from therapy. With the utilization of the Choi Criteria in this study, we identified a response rate of 42% with additional 11% having stable disease. Furthermore, use of these response criteria for treatment decisions resulted in an improvement in overall survival compared to historical controls supporting both that the Choi Criteria appropriately identified treatment responders and that axitinib is an effective therapeutics in heavily pretreated R/M HNSCC.

Targeted therapy has the demonstrated promise in pre-clinical studies in HNSCC. Alterations in PI3KCA, CDKN2A, and EGFR suggest head and neck cancer being a candidate for development of targeted therapeutics. However, this approach has had limited clinical success. The only approved agent, cetuximab, has been demonstrated to improve survival by less than three months[18, 19]. Tyrosine kinase inhibitors offer the benefit of targeting numerous pathways (ie VEGFR, EGFR, PDGFR) and isoforms simultaneously. Axitinib has been demonstrated to inhibit VEGFR (-1,-2, and -3) as well as c-Kit. Mounting evidence suggest that VEGF inhibition is immunomodulatory via numerous mechanisms including production of IFNγ, reversal of the immunosuppressive microenvironment, and augmented activity of CD8+ T cells via hypoxia-inducible factor-1α secondary to tumor hypoxia[20-22]. As VEGFR inhibition may prime the immune system for response to immunotherapy, sequential use may be a modality to decrease toxicities yet still gain therapeutic synergy. In the small subgroup of patients that were treated with immunotherapy following axitinib (n=11), the RR to PD-1 monotherapy was 45% including one patient with a complete response. Although conclusions cannot be drawn given the limited sample size, previous trials have shown a RR of 13-17% in biomarker unselected populations[4, 23, 24] hence supporting possible potentiation with sequential therapy. Preliminary results from the phase Ib/II KEYNOTE-526 trial evaluating concurrent lenvatinib (an inhibitor of VEGFR 1-3, FGFR 1-4, and PDGFRα) and pembrolizumab demonstrated a response rate of 40.9% and median PFS of 8.2 months supporting further investigation of this combination[25].

57% of patients in this study experienced grade 3 or 4 toxicities of which the most common was fatigue. Large studies of single agent treatment regimens employed in this patient population have demonstrated toxicity rates ranging from 35-46%[18, 24]. Although this study has a higher rate of serious toxicities relative to comparable agents, our previous study of single agent axitinib demonstrated a much lower rate of severe toxicities (40%)[12]. The toxicities encountered were manageable with dose reductions supporting patient tolerability. As aforementioned, there is promise of significant synergy with the combination of VEGF inhibition and PD-1 inhibition. Ongoing phase 3 trials are evaluating concurrent lenvatinib and pembrolizumab in patients with PD-L1 $\geq$ 1%. However, preliminary reports of clinical trials evaluating this combination describe grade 3 or 4 toxicities in 91% of patients and leading to 18% of study participants discontinuing treatment. Sequential therapy (ie axitinib followed by single agent immune checkpoint inhibitor) may offer a way to prime the immune system hence obtaining a synergistic response without encountering severe toxicities. This approach merits further clinical investigation.

The treatment paradigm and anticipated survival for patients with R/M HNSCC is rapidly changing. KEYNOTE-048 demonstrated a median overall survival of 12.3 months for patients with head and neck cancer treated with first line immunotherapy. However, this study included exclusively newly diagnosed platinum sensitive disease[4]. A more appropriate contemporary comparator population for this study is the CheckMate-141 trial evaluating nivolumab in platinum refractory R/M HNSCC of which 55% of patients had greater than one previous line of systemic therapy. In this trial, the median overall survival in patients treated with nivolumab was 7.5 months versus 5.1 months in patients treated with standard of care chemotherapy[24]. Our study demonstrates a median overall survival of 9.8 months in a heavily pretreated population of which 61% received greater than one line of systemic therapy, 61% of patients were platinum refractory, and 42% of patients were refractory or PD-1 inhibitors.

This result is surprising due to the complex array of genetic alterations observed in advanced HNSCC patients. For example, through the available genomic data in this study, we identified two patients with tumors containing *KDR* (*VEGFR2*) mutations. Unfortunately, the functional significance of these alterations are currently unknown, even though this understanding would be important to help elucidate whether the positive effects of axitinib were due to function on tumor cells or to supporting cells in the microenvironment. For example, because one of these patients responded to therapy, if the *KDR* mutations are found to be activating and sufficient to make the protein resistant to axitinib, then the clinical data would suggest that inhibition of VEGF/VEGFR signaling in the tumor microenvironment may be more critical than inhibition of *KDR* signaling in tumor cells. As such, this trial opens an exciting area of research related to the pivotal for of VEGF/VEGFR signaling in HNSCC.

Importantly, we also the first to report a clinical link between PI3K status and response to axitinib. Given that approximately 45% of HNSCC harbor PI3K pathway alterations, future studies are warranted to evaluate whether PI3K pathway alterations are predictive of response to axitinib and potential mechanistic links between the two pathways in HNSCC. Multiple potential mechanisms may account for the relationship, for example, tumors with PI3K alterations are often induce angiogenesis through VEGF-regulated cytokine mechanisms, and perhaps this process is critical for the survival of PI3K-dependent tumors[26]. While future studies are necessary to help dissect the relationship between these two pathways, our discovery has the potential for profound clinical impact in this patient population and should be evaluated in larger patient cohorts.

Although our study supports the activity of axitinib in heavily pretreated R/M HNSCC, there are limitations. The population was somewhat heterogeneous both in sites of primary disease and previous treatments. Patients with cutaneous squamous cell carcinoma (cSCC) are often excluded from R/M HNSCC given a distinct disease course and longer survival[27, 28]. To evaluate this potential confounding factor, we evaluated the survival of the six patients with cSCC and found they had a worse OS, although not statistically significant, compared to the non-cSCC patients, in keeping with the low response rate within this subgroup. Hence, we do not believe this limits the interpretation of our results. Finally, given the improvement in OS with use of PD-1 inhibitors in heavily pretreated R/M HNSCC and the fact that only 11 patients (39.2%) were treated with a checkpoint inhibitor prior to enrollment, we questioned the role of potential receipt of a PD-1 inhibitor as a subsequent line of therapy as influencing survival within our study population. Eleven patients received a PD-1 inhibitor as some line of therapy after progression on axitinib. Exploratory analysis demonstrated no difference in survival between those subsequently treated with a checkpoint inhibitor versus those who were not suggesting this was not a confounding variable. Uniform inclusion criteria for previous treatments should be employed evaluating VEGF inhibition in futures studies.

Conclusion

Axitinib treatment is associated with improved survival in patients with heavily pre-treated head and neck cancer. The Choi Criteria were able to classify treatment responses amongst patients with an atypical radiographic response and should be considered for use in future trials of VEGFR directed tyrosine kinase inhibitors in head and neck cancer. Exploratory analysis suggests that marked response rates are seen with the use of a single agent ICI after axitinib (RR: 45%) and patients with PI3K pathway alterations may derive exceptional benefit from therapy (RR: 75% vs. 17%). Further investigation is warranted to evaluate its activity in biomarker selected populations, especially as a mechanism to prime the immune microenvironment prior to immune checkpoint inhibitor therapy.

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Tables

Table 1: Patient Demographics and Clinical Characteristics

This table describes the baseline demographics of the patients included in analysis for efficacy.

Age	n	28
	Mean	63.9
	Median (range)	64.5 (37-80)
Gender, n (%)	Male	25 (89%)
	Female	3 (11%)
ECOG Performance Status, n (%)	0 (Fully functional)	11 (39%)
	1 (Minor Impairment)	17 (61%)
Disease Primary Site, n (%)	Oral Cavity	2 (7.1%)
	Oropharynx	13 (46%)
	Larynx	4 (13.3%)
	Nasopharynx	3 (10.7%)
	Cutaneous	6 (21.4%)
HPV Status, n (%)	Positive	10 (35.7%)
	Negative	17 (60.7%)
	Unknown	1 (3.6%)
Previous Lines of Therapy	0	11 (39%)
	1	6 (21.4%)
	2	5 (17.8%)
	3+	6 (21.4%)
Previous Exposure to Platinum	Sensitive	11 (39.2%)
	Refractory	17 (60.7%)

Previous Exposure to PD-1 Inhibitor	n	11 (39.2%)
	Primary Resistant	3 (27%)
	Acquired Resistance	8 (54%)

Table 2: Treatment Related Toxicities

This table demonstrates the toxicities observed in the entire study population (n=29) with a frequency of greater than 10%

Toxicity	**Grade 1 or 2**	**Grade 3 or 4**	**All Grades**
Fatigue	15 (54%)	6 (21%)	21 (75%)
Hypertension	13 (46%)	2 (7%)	15 (54%)
Oral Mucositis	2 (7%)	2 (7%)	4 (14%)
Diarrhea	6 (21%)	1 (4%)	7 (25%)
Oral pain	2 (7%)	1 (4%)	3 (11%)
Bleeding	4 (14%)	1 (4%)	5 (18%)
Nausea	9 (32%)	0 (0%)	9 (32%)
Weight loss	7 (25%)	0 (0%)	7 (25%)
Anorexia	6 (21%)	0 (0%)	6 (21%)
Aspartate aminotransferase increased	6 (21%)	0 (0%)	6 (21%)
Dysgeusia	5 (18%)	0 (0%)	5 (18%)
Vomiting	5 (18%)	0 (0%)	5 (18%)
Hoarseness	4 (14%)	0 (0%)	4 (14%)
Sore throat	4 (14%)	0 (0%)	4 (14%)
Dehydration	3 (11%)	0 (0%)	3 (11%)

Table 3: Treatment Efficacy

This table describes the efficacy and outcomes among A) evaluable patients and b) those with sequencing results

A	
6 month PFS (95% CI)[1]	32% (18%,51%)
Median PFS, days (95% CI)[1]	107.5 (72-164)
	3.5 months
6 month OS (95% CI)[1]	71% (53%, 85%)
Median OS, days KM estimate (95% CI)[1]	301 (182,372)
	9.8 months
Best Overall Response Rate	42%
Progressive Disease (PD), n (%)	10 (36%)
Stable Disease (SD), n (%)	3 (11%)
Partial Response (PR), n (%)	11 (39%)
Complete Response (CR), n(%)	1 (3%)
Off Treatment before 8 week scan, n (%)	3 (11%)

1- 6 month survival proportion and 95% confidence interval estimated using Wilson score interval method.

B		
	Response Rate (# responders/patients)	
	Mutant	Wild-Type
PI3K Signaling Pathway Alterations	75% (6/8)	17% (2/12)
Non-Cutaneous Squamous Cell Carcinoma	86% (6/7)[A]	12% (1/8)
Cutaneous Squamous Cell Carcinoma	0% (0/1)	25% (1/4)[B]
KMT2C/D Mutations	33% (2/6)	50% (6/12)
Non-Cutaneous Squamous Cell Carcinoma	66% (2/3)[C]	50% (5/10)
Cutaneous Squamous Cell Carcinoma	0% (0/3)	50% (1/2)[B]

[A] Remaining patient had SD as best response to therapy.

[B] Patient had a KDR (VEGFR2) S110F mutation and exhibited a complete response

[C] Both patients who exhibited a response had synchronous mutations in the PI3K signaling pathway

Figure Legends

Figure 1: Kaplan Meier Survival Analysis- These figures illustrates the overall survival (1a) and progression free survival (1b) amongst patients treated with axitinib

Figure 2: Degree of Tumor Response- These figures demonstrate the maximal degree of response to treatment by Choi Criteria amongst evaluable patients (2a) as well as those with genomic sequencing results, clustered by mutation status (2b)

Figure 3: Genomic Alterations of Patient Cohort- This figure illustrates alteration status of selected genes of interest amongst evaluable patients with sequencing results.

Original Article

Axitinib in Head and Neck Cancer/Swiecicki et al

Efficacy of Axitinib in Metastatic Head and Neck Cancer With Novel Radiographic Response Criteria<zaq;1>

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<FNTX>Additional supporting information may be found online in the Supporting Information section at the end of the article.

We are grateful to the patients and families, without whom this study would not have been possible. We are particularly appreciative of the clinical and research staff assisting with the care of these patients.

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BACKGROUND: There are limited treatment options for unresectable recurrent or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC). Vascular endothelial growth factor is of significant interest for targeted therapy in R/M HNSCC because of its central role in tumorigenesis and immunosuppression. Axitinib is a potent inhibitor of vascular endothelial growth factor receptor 1 (VEGFR1), VEGFR2, VEGFR3, platelet-derived growth factor receptor<zaq;3>, and c-kit<zaq;4> and offers such an approach.

METHODS: This article reports the results of a phase 2 trial evaluating axitinib in R/M HNSCC according to the Choi criteria for radiographic response assessment.<zaq;5> The primary endpoint of this trial was 6-month overall survival.

RESULTS: Twenty-nine patients were enrolled, and 28 were evaluable for a response. Patients were heavily pretreated, with 61% having had at least 1 previous systemic treatment in the metastatic setting (range, 0-5). The median overall survival of 9.8 months and the 6-month overall survival rate of 70%<zaq;3> met the protocol-defined criteria for clinical efficacy. The best overall response rate was 42%. Correlative analyses demonstrated that PI3K signaling pathway alterations were associated with an increased response to therapy (75% vs 17%). A marked response to therapy was seen in a subgroup of patients who were treated with an immune checkpoint inhibitor after progression on axitinib.

CONCLUSIONS: Treatment with axitinib is associated with improved survival in patients with heavily pretreated head and neck cancer, and PI3K pathway alterations may serve as a biomarker for response. Further investigation is warranted to evaluate axitinib in biomarker-selected populations, especially in combination with immune checkpoint inhibitor therapy.

LAY SUMMARY:<zaq;6>

• Metastatic head and neck squamous cancer is an incurable disease with limited treatment options and a poor prognosis.

• This study is the first to demonstrate that the targeted oral drug axitinib improves survival in patients with heavily pretreated metastatic head and neck cancer.

• Furthermore, patients whose tumors have specific mutations derive the greatest benefit from therapy.

• The investigation of axitinib alone or in combination with immunotherapy in a genomic biomarker–selected population is warranted.

KEYWORDS: axitinib, Choi criteria, head and neck cancer, PI3K, vascular endothelial growth factor receptor inhibitor.

<H1>INTRODUCTION</H1>

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with 600,000 new cases worldwide each year, and the incidence rate is increasing at an unprecedented rate because of the high prevalence of human papillomavirus–induced HNSCC.[1] In fact, oropharyngeal cancer is 1 of only 4 cancers increasing in incidence in the United States.[2] Although the majority of patients with HNSCC are cured with multimodality therapy, a significant proportion of patients develop unresectable recurrent or metastatic (R/M) HNSCC. Despite the recent development of programmed death 1 (PD-1) inhibitors, response rates remain low because of variability within the immune microenvironment.[3] Even with these novel therapies, the median survival for patients newly diagnosed with R/M HNSCC is approximately 12 months.[4]

With increasing molecular characterization of HNSCC, there has been significant interest in targeted therapy.[5] Vascular endothelial growth factor (VEGF) dysregulation has been identified as a crucial process in R/M HNSCC in not only angiogenesis but also progression, immunosuppression, and immune tolerance.[6,7] Furthermore, VEGF overexpression is associated with advanced disease and a poor prognosis.[8,9] Because of this central role in advanced disease and tumorigenesis, VEGF inhibition is of significant interest as a candidate for targeted therapy.

Axitinib is a multireceptor tyrosine kinase inhibitor<zaq;7> approved for renal cell carcinoma that inhibits several isoforms of the VEGF receptor (vascular endothelial growth factor receptor 1 [VEGFR1], VEGFR2, and VEGFR3). Furthermore, it has inhibitory activity against platelet-derived growth factor receptor (PDGFR)<zaq;3> and downstream effectors of epidermal growth factor receptor (EGFR), both of which are commonly disrupted and contribute to head and neck tumorigenesis.[5,10,11] Because of this mechanism of action and known molecular alterations in R/M HNSCC, it seems to be a promising agent for clinical assessment.

We previously reported a phase 2 study evaluating axitinib in patients with heavily pretreated R/M HNSCC. This work demonstrated a low response rate (7%) with single-agent axitinib; however, a significant proportion of patients had stable disease (70%) with radiographic findings consistent with a treatment response.[12] Moreover, the population had impressive overall survival (10.9 months), which suggested that efficacy was perhaps not captured. Hence, we postulated that axitinib held significant antitumor activity in R/M HNSCC, but the Response Evaluation Criteria in Solid Tumors (RECIST) failed to appropriately capture responders and may have inappropriately suggested tumor progression. Differential manifestations of response have been seen with the use of tyrosine kinase inhibitors (ie, swelling and cystic attenuation) that have the potential of being wrongly interpreted<zaq;8> as progressive disease by RECIST, and this prompted the development of the Choi criteria.[13]

On the basis of these findings, we initiated a new follow-up phase 2 study to investigate the clinical activity of axitinib in R/M HNSCC with the Choi criteria for response assessment. Our hypothesis was that axitinib would have significant antitumor activity as judged by the Choi criteria and would result in an improvement in the 6-month overall survival in comparison with a historical control.

<H1>MATERIALS AND METHODS</H1>

<H2>Patient Eligibility</H2>

This was a phase 2, open-label trial approved by the institutional review board of the University of Michigan Rogel Cancer Center (NCT02762513). All patients provided written informed consent. Patients 18 years old or older with histologically documented unresectable R/M HNSCC were eligible. All patients were required to have measurable disease according to a computed tomography scan or cutaneous lesions ≥ 10 mm that were not assessable on imaging but were present on physical examination, an Eastern Cooperative Oncology Group performance status of 0 to 2, and a life expectancy ≥ 12 weeks. Adequate hematopoietic, hepatic, and renal function was required, and this was defined as an absolute neutrophil count ≥ 1.5 × 10^9 cells/mL, a platelet count ≥ 75,000 cells/mm^3, a hemoglobin level ≥ 9.0 g/dL,<zaq;3> a total serum bilirubin concentration within 1.5 times the upper limit of normal, aspartate aminotransferase and alanine aminotransferase concentrations within 2.5 times the institutional upper limits of normal (unless there were liver metastases, in which case the aspartate aminotransferase and alanine aminotransferase concentrations had to be within 5.0 times the upper limit of normal), a serum creatinine clearance ≥ 30 mL/min, and a urinary protein level < 2+.

Women of childbearing potential must have had a negative serum or urine pregnancy test within the 3 days before treatment.

Patients who had tumors encasing major blood vessels or active hemoptysis (>0.5 teaspoons of bright red blood per day) or were currently using therapeutic anticoagulation were excluded, as were those with gastrointestinal abnormalities resulting in impaired absorption. Treatment with EGFR inhibitors within the 30 days preceding study entrance was prohibited. Patients were excluded if they had uncontrolled hypertension before enrollment, which was defined as a systolic blood pressure reading > 140 mm Hg and/or a diastolic blood pressure reading > 90 mm Hg.

<H2>Treatment Plan</H2>

Enrolled patients underwent a complete history and physical examination, baseline laboratory studies (complete blood count with differential, comprehensive metabolic profile, thyroid-stimulating hormone, and urinalysis),<zaq;9> and radiographic staging studies (neck/chest computed tomography and others as clinically warranted). If cutaneous lesions were not assessable for a response by imaging, pictures of the target lesions were obtained as well. All screening assessments were completed within the 28 days before the start of treatment.

Patients were initiated on axitinib at 5 mg twice daily with a cycle length of 28 days.<zaq;10> Dose escalation was planned at 2 weeks (to 7 mg twice daily) and 3 weeks for a goal of 10 mg twice daily in the absence of grade 2 or higher toxicities. Patients were seen for toxicity and laboratory assessments (complete blood count, comprehensive metabolic panel, thyroid-stimulating hormone, and urinalysis)<zaq;11> at 2 and 4 weeks and then monthly after treatment initiation. Response to therapy.<zaq;12> Dose escalation could be resumed at the next visit if toxicities diminished to grade 1 or lower. Treatment was continued until disease progression, unacceptable toxicity, or patient withdrawal of consent or at the discretion of the investigator.

<H2>Evaluation of Response</H2>

A response assessment was performed after 2 cycles of axitinib treatment, and this was continued every 2 cycles. Radiographic assessments obtained at enrollment were obtained at each time point. Similarly, if a physical examination was being used for the response assessment of cutaneous lesions, pictures were taken at each time point.

Photographs as well as imaging studies were submitted to the University of Michigan Tumor Response and Assessment Core. The radiologic response was determined according to the Choi criteria.[13]

<H2>Statistical Considerations</H2>

Twenty-nine patients were enrolled between August 30, 2016, and October 23, 2019. The median follow-up duration among the study participants was 18 months (range, 1-36 months), and no patients remained on therapy. Follow-up for patients still living ranged from 5 to 32 months. On the basis of our previous study supporting an improvement in survival for patients with R/M HNSCC treated with axitinib, we designed this expansion study. Although consideration was given to adjusting this original study to a Bayesian expansion trial design, it was ultimately decided<zaq;3> to begin a new cohort to test for an improvement in survival under the same mortality rate assumptions used in the previous study. Notably, treatment continuation decisions for this trial were based on Choi criteria that, as previously reported,[12] considerably differed from RECIST decisions when evaluated in the original trial.

The primary aim was to compare 6-month overall survival after treatment with axitinib in patients with unresectable R/M head and neck cancer with historical rates. On the basis of results in the literature, we assumed a 6-month mortality rate of 50% under current standard care in this patient population.[14] A sample size of 37 patients was planned to test whether survival after treatment with axitinib was improved to 70% at 6 months in comparison with 50% with an upper tailed test of binomial proportion. No interim analyses for activity were planned. Because of an observed clinical benefit and a slowed accrual rate, an unplanned interim analysis was performed after the enrollment of 29 patients. Data were analyzed by the study statistician; a statistically meaningful improvement in survival was identified in this analysis, and the decision was made to close the study to further accrual.

Overall survival was defined as the time from study enrollment to death from any cause. Six-month overall survival was defined as the proportion of patients who received at least 1 cycle of axitinib<zaq;3> and were alive 6 months after study enrollment, and 95% confidence intervals (CIs) were estimated with the Wilson score interval method. Treatment-related adverse events were graded according to the Common Terminology

Criteria for Adverse Events, version 4.03. The response rate was defined as the sum of patients with complete responses and partial responses according to the Choi criteria. Statistical analysis was performed with SAS v14.3 software (SAS, Carey, North Carolina). Planned correlative analyses included a genomic analysis of patients when next-generation sequencing results were evaluable.

<H1>RESULTS</H1>

<H2>Patient Characteristics</H2>

Twenty-nine patients were enrolled, 1 of whom died before treatment with axitinib. All 28 patients who received at least 1 dose of axitinib were included for the toxicity analysis; the baseline characteristics are summarized in Table 1. The mean age was 63.9 years (range, 37-80 years), and the majority of the patients (61% [n = 17]) had an Eastern Cooperative Oncology Group performance score of 1, which indicated mild impairment. The primary site of disease for most patients was the oropharynx (42.6% [n = 13]),<zaq;13> and the majority of the study participants were negative for human papillomavirus (60.7% [n = 17]). The majority of the patients (61% [n = 17]) had at least 1 previous systemic treatment in the metastatic setting, with the number of previous lines of treatments ranging from 0 to 5. Seventeen patients (61%) were refractory to platinum therapy (defined as progression within 180 days of chemotherapy), and 12 patients (43%)<zaq;14> were previously treated with a PD-1 inhibitor.

<H2>Toxicity</H2>

The median duration of treatment was 3 cycles (range, 1-9 cycles).The most common toxicities included fatigue (75%), hypertension (54%), nausea (32%), and diarrhea (25%; Table 2). Bleeding was observed in 5 patients, including 1 patient with a grade 3 lower gastrointestinal bleed; all cases spontaneously resolved and recurred with the re-initiation of axitinib. Grade 3 or 4 severe toxicities were seen in 16 patients (57%). Severe toxicities included fatigue (21%), hypertension (7%), and mucositis (7%). No grade 5 events were reported. Overall, the observed toxicities were consistent with those previously reported in the literature.[15,16]

<H2>Efficacy</H2>

The 6-month overall survival rate was 71% (95% CI, 53%-85%; Table 3). This met the protocol-defined criteria for supporting evidence of clinical benefit. The median progression-free survival was 3.5 months (95% CI, 2.4-5.4 months), and the median overall survival was 9.8 months (95% CI, 5.9-12.2 months; Fig. 1).

Three patients completed their trial participation before response imaging: one on account of adverse effects (but the patient was clinically noted to have progressive disease),<zaq;3> another on account of death due to progressive disease, and a third on account of withdrawal from the study. The overall response rate was 42%,<zaq;15> and the disease control rate was 53%. The waterfall plot in Figure 2A graphically demonstrates the depth of response among participants evaluable for a response. One patient had a durable complete response. Only 1 patient with cutaneous squamous cell carcinoma demonstrated a response to therapy. This patient had a mutation in KDR (VEGFR2) and achieved a durable complete response. All of the remaining 6 patients with cutaneous squamous cell carcinoma had progressive disease.

Given the immunomodulatory potential of VEGFR inhibition, we evaluated the treatment response in patients who received PD-1 inhibition as part of their treatment course. Eleven patients were treated with a PD-1 inhibitor before treatment with axitinib. Three patients had primary resistance to checkpoint inhibitor therapy, and none of these patients responded to axitinib (0 of 3). Eight had acquired resistance to checkpoint inhibitor therapy: 3 of these patients had a partial response with axitinib (3 of 8 [37%]<zaq;16>), 2 had stable disease (2 of 8 [25%]), and 3 had progressive disease (3 of 8 [37%]). Eleven patients were treated with a PD-1 inhibitor after progression on axitinib with an observed response rate of 45% (5 of 11). The response assessment demonstrated a complete response in 1 patient (1 of 11 [9%]), a partial response in 4 patients (4 of 11 [36%]), and stable disease in 1 patient (1 of 11 [9%]); progressive disease was seen in the remaining 5 patients (5 of 11 [45%]).

<H2>Correlative Studies</H2>

To evaluate the association between genomic alterations, tumor characteristics, and clinical outcomes, we analyzed results from patients who had commercial next-generation sequencing previously performed (n = 20). The investigators defined a set of genes (sequenced as part of all next-generation sequencing panels), and recurrent alterations are shown (Fig. 3). Importantly, although no mutations were identified in FLT1 (VEGFR1), FLT4 (VEGFR3), PDGFR, or KIT, 2 patients had mutations<zaq;3> in

KDR (VEGFR2), including an S1100F mutation as well as 2 mutant alleles (R1032Q and G638R; Supporting Table 1). The ability of axitinib to inhibit these mutant forms of KDR is unknown; however, the patient with the S110F mutation had a complete response, whereas the other had progressive disease. Importantly, 55% of the patients (11 of 20) had TP53 alterations; 40% of the patients (8 of 20) harbored alterations to genes in the PI3K pathway, including PTEN and PIK3CA; and 30% of the patients (6 of 20) had mutations in either KMT2C (MLL2) or KMT2D (MLL3).

The degree of response and the pathway alterations were correlated for an exploratory analysis (Fig. 2B). The relative response rate<zaq;17> was 75% for patients with mutations in the PI3K pathway and 39% for wild-type patients (6 of 8 patients vs 2 of 12 patients). In terms of the KMT2C/D pathway, the response rate was 33% in the mutant population and 50% in the rest of the population (2 of 6 patients vs 6 of 12 patients).

Because of the differential responses seen between patients with cutaneous squamous cell carcinoma and patients with noncutaneous primaries, the response rates were further explored (Table 3). Although sample sizes were limited, mutations in the PI3K pathway were associated with a higher response rate in comparison with the wild-type population in noncutaneous squamous cell carcinomas (86% vs 12%).

<H1>DISCUSSION</H1>

In this phase 2 study of patients with heavily pretreated unresectable R/M HNSCC, axitinib demonstrated an improvement in 6-month overall survival in comparison with historical controls (70% vs 50%). Furthermore, treatment resulted in significant response rates and lower rates of severe toxicities.

There is increasing recognition of variable radiographic manifestations of response with the advent of novel classes of therapeutics. Most recognized is the “pseudoprogression” observed with immunotherapy, which prompted the development of iRECIST to capture atypical responses.[17] The Choi response criteria have been best evaluated in gastrointestinal stromal tumors, for which, in comparison with RECIST, they have been demonstrated to better predict survival.[13] In our previous trial using RECIST, we observed a low RECIST-assessed objective response rate but paradoxically high and impressive overall survival among heavily pretreated patients.[12] As such, we hypothesized that we were underappreciating treatment responses with the use of

RECIST, and the Choi criteria may be more appropriate for discerning those patients benefiting from therapy. With the utilization of the Choi criteria in this study, we identified a response rate of 42%, with an additional 11% having stable disease. Furthermore, the use of these response criteria for treatment decisions resulted in an improvement in overall survival in comparison with historical controls, and this supports both that the Choi criteria appropriately identified treatment responders and that axitinib is an effective therapy for heavily pretreated patients with R/M HNSCC.

Targeted therapy has demonstrated promise in preclinical studies of HNSCC. Alterations in PI3KCA, CDKN2A, and EGFR suggest that head and neck cancer is a candidate for the development of targeted therapeutics. However, this approach has had limited clinical success. The only approved agent, cetuximab, has been demonstrated to improve survival by less than 3 months.[18,19] Tyrosine kinase inhibitors offer the benefit of targeting numerous pathways (ie, VEGFR, EGFR, and PDGFR) and isoforms simultaneously. Axitinib has been demonstrated to inhibit VEGFR1, VEGFR2, and VEGFR3<zaq;18> as well as c-Kit. Mounting evidence suggests that VEGF inhibition is immunomodulatory via numerous mechanisms, including the production of interferon γ, reversal of the immunosuppressive microenvironment, and augmented activity of CD8+ T cells via hypoxia-inducible factor 1α secondary to tumor hypoxia.[20-22] Because VEGFR inhibition may prime the immune system for a response to immunotherapy, sequential use may be a modality to decrease toxicities yet still gain therapeutic synergy. In the small subgroup of patients who were treated with immunotherapy after axitinib (n = 11), the response rate<zaq;19> to PD-1 monotherapy was 45%; this included 1 patient with a complete response. Although conclusions cannot be drawn because of the limited sample size, previous trials have shown response rates<zaq;19> of 13% to 17% in biomarker-unselected populations,[4,23,24] so this supports possible potentiation with sequential therapy. Preliminary results from the phase 1b/2 KEYNOTE-526 trial evaluating concurrent lenvatinib (an inhibitor of VEGFR1, VEGFR2, VEGFR3, FGFR1, FGFR2, FGFR3, FGFR4, and PDGFRα)<zaq;3> and pembrolizumab demonstrated a response rate of 40.9% and median progression-free survival of 8.2 months, which supported further investigation of this combination.[25]

Fifty-seven percent of the patients in this study experienced grade 3 or 4 toxicities, the most common of which was fatigue. Large studies of the single-agent treatment regimens used in this patient population have demonstrated toxicity rates ranging from 35% to 46%.[18,24] Although this study has a higher rate of serious toxicities in comparison with comparable agents, our previous study of single-agent axitinib demonstrated a much lower rate of severe toxicities (40%).[12] The toxicities encountered were manageable with dose reductions, and this supports patient tolerability. As previously mentioned, there is a promise of significant synergy with the combination of VEGF inhibition and PD-1 inhibition. Ongoing phase 3 trials are evaluating concurrent lenvatinib and pembrolizumab in patients with PD-L1 ≥ 1%<zaq;20>. However, preliminary reports of clinical trials evaluating this combination describe grade 3 or 4 toxicities in 91% of patients, and this leads to 18% of study participants discontinuing treatment. Sequential therapy (ie, axitinib followed by a single-agent immune checkpoint inhibitor) may offer a way to prime the immune system and hence obtain a synergistic response without encountering severe toxicities. This approach merits further clinical investigation.

The treatment paradigm and anticipated survival for patients with R/M HNSCC are rapidly changing. KEYNOTE-048 demonstrated a median overall survival of 12.3 months for patients with head and neck cancer treated with first-line immunotherapy. However, this study exclusively included newly diagnosed platinum-sensitive disease.[4] A more appropriate contemporary comparator population for this study is the CheckMate-141 trial, which evaluated nivolumab in platinum-refractory R/M HNSCC; 55% of the patients had more than 1 previous line of systemic therapy. In this trial, the median overall survival was 7.5 months for patients treated with nivolumab and 5.1 months for patients treated with standard-of-care chemotherapy.[24] Our study demonstrated a median overall survival of 9.8 months in a heavily pretreated population in which 61% received more than 1 line of systemic therapy, 61% were refractory to platinum, and 42% were refractory to PD-1 inhibitors.<zaq;21>

This result is surprising because of the complex array of genetic alterations observed in patients with advanced HNSCC. For example, through the genomic data available in this study, we identified 2 patients with tumors containing KDR (VEGFR2) mutations. Unfortunately, the functional significance of these alterations is currently unknown, even though this understanding would be important for elucidating whether the positive effects of axitinib are due to its function on tumor cells or supporting cells<zaq;22> in the microenvironment. For example, because 1 of these patients responded to therapy, if the KDR mutations are found to be activating and sufficient to make the protein resistant to axitinib, then the clinical data would suggest that inhibition of VEGF/VEGFR signaling in the tumor microenvironment may be more critical than inhibition of KDR signaling in tumor cells. As such, this trial opens an exciting area of research related to the pivotal for of VEGF/VEGFR signaling in HNSCC.

Importantly, we are also the first to report a clinical link between the PI3K status and the response to axitinib. Because approximately 45% of HNSCCs harbor PI3K pathway alterations, future studies are warranted to evaluate whether PI3K pathway alterations are predictive of a response to axitinib and potential mechanistic links between the 2 pathways in HNSCC. Multiple potential mechanisms may account for the relationship; for example, tumors with PI3K alterations often induce angiogenesis through VEGF-regulated cytokine mechanisms, and perhaps this process is critical for the survival of PI3K-dependent tumors.[26] Although future studies are necessary to help to dissect the relationship between these 2 pathways, our discovery has the potential for a profound clinical impact on this patient population and should be evaluated in larger patient cohorts.

Although our study supports the activity of axitinib in heavily pretreated R/M HNSCC, there are limitations. The population was somewhat heterogeneous in both sites of primary disease and previous treatments. Patients with cutaneous squamous cell carcinoma are often excluded from studies of R/M HNSCC<zaq;3> because of the distinct disease course and longer survival.[27,28] To evaluate this potential confounding factor, we evaluated the survival of the 6 patients with cutaneous squamous cell carcinoma and found that they had worse overall survival, although this was not statistically significant, in comparison with the patients with noncutaneous squamous cell carcinoma<zaq;23>, and this was in keeping with the low response rate within this subgroup. Hence, we do not believe that this limits the interpretation of our results. Finally, given the improvement in overall survival with the use of PD-1 inhibitors in heavily pretreated R/M HNSCC and given the fact that only 11 patients (39.2%)<zaq;24>

were treated with a checkpoint inhibitor before enrollment, we questioned the role of the potential receipt of a PD-1 inhibitor as a subsequent line of therapy in influencing survival within our study population. Eleven patients received a PD-1 inhibitor as some line of therapy after progression on axitinib. An exploratory analysis demonstrated no difference in survival between those subsequently treated with a checkpoint inhibitor and those who were not, and this finding suggested that this was not a confounding variable. Uniform inclusion criteria for previous treatments should be used for evaluating VEGF inhibition in future studies.

In conclusion, axitinib treatment is associated with improved survival in patients with heavily pretreated head and neck cancer. The Choi criteria were able to classify treatment responses among patients with an atypical radiographic response and should be considered for use in future trials of VEGFR-directed tyrosine kinase inhibitors in head and neck cancer. An exploratory analysis suggests that marked response rates are seen with the use of a single-agent immune checkpoint inhibitor after axitinib (response rate<zaq;19>, 45%), and patients with PI3K pathway alterations may derive an exceptional benefit from therapy (response rate<zaq;19>, 75% vs 17%). Further investigation is warranted to evaluate its activity in biomarker-selected populations, especially as a mechanism for priming the immune microenvironment before immune checkpoint inhibitor therapy.

<H1>FUNDING SUPPORT</H1><zaq;25>

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<H1>CONFLICT OF INTEREST DISCLOSURES</H1>

Paul L. Swiecicki and J. Chad Brenner have applied for provisional patents (not awarded yet) for companion diagnostics related to the data presented in this publication. Swiecicki also reports grants from Ascentage Pharma and personal fees from Regeneron outside the submitted work. Francis P. Worden reports personal fees from Merck, Bristol-Myers Squibb, and Regeneron outside the submitted work. The other authors made no disclosures.

<H1>AUTHOR CONTRIBUTIONS</H1>

Paul L. Swiecicki: Conceptualization, data curation, formal analysis, methodology, resources, manuscript writing, and manuscript editing. **Emily L. Bellile:** Data curation, formal analysis, methodology, manuscript writing, and manuscript editing. **Collin Brummel:** Data curation, formal analysis, and manuscript editing. **J. Chad Brenner:** Formal analysis, methodology, manuscript writing, and manuscript editing. **Francis P. Worden:** Conceptualization, resources, and manuscript editing.

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Figure 1. Kaplan-Meier survival analysis. This figure illustrates (A) overall survival and (B) progression-free survival among patients treated with axitinib.

Figure 2. Degree of tumor response. This figure demonstrates the maximal degree of response to treatment by the Choi criteria among (A) evaluable patients and (B) those with genomic sequencing results clustered by the mutation status. CR indicates complete response; PD, progressive disease; PR, partial response; SD, stable disease.<zaq;26>

Figure 3. Genomic alterations in the patient cohort. This figure illustrates the alteration status of selected genes of interest among evaluable patients with sequencing results.

TABLE 1. Patient Demographics and Clinical Characteristics

Age (n = 28), y<zaq;27>	
Mean	63.9
Median (range)	64.5 (37-80)
Sex, No. (%)	
Male	25 (89)
Female	3 (11)
ECOG performance status, No. (%)	
0 (fully functional)	11 (39)
1 (minor impairment)	17 (61)
Disease primary site, No. (%)	
Oral cavity	2 (7.1)
Oropharynx	13 (46)
Larynx	4 (13.3)
Nasopharynx	3 (10.7)
Cutaneous	6 (21.4)
HPV status, No. (%)	
Positive	10 (35.7)
Negative	17 (60.7)
Unknown	1 (3.6)
Previous lines of therapy, No. (%)	
0	11 (39)
1	6 (21.4)
2	5 (17.8)
≥3	6 (21.4)
Previous exposure to platinum, No. (%)	
Sensitive	11 (39.2)
Refractory	17 (60.7)
Previous exposure to PD-1 inhibitor (n = 11 [39.2%]), No. (%)	
Primary resistant	3 (27)
Acquired resistance	8 (54)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HPV, human papillomavirus; PD-1, programmed death 1.

This table describes the baseline demographics of the patients included in the analysis for efficacy.

TABLE 2. Treatment-Related Toxicities<zaq;28>

Toxicity	Grade 1 or 2, No. (%)	Grade 3 or 4, No. (%)	All Grades, No. (%)
Fatigue	15 (54)	6 (21)	21 (75)
Hypertension	13 (46)	2 (7)	15 (54)
Oral mucositis	2 (7)	2 (7)	4 (14)
Diarrhea	6 (21)	1 (4)	7 (25)
Oral pain	2 (7)	1 (4)	3 (11)
Bleeding	4 (14)	1 (4)	5 (18)
Nausea	9 (32)	0 (0)	9 (32)
Weight loss	7 (25)	0 (0)	7 (25)

Anorexia	6 (21)	0 (0)	6 (21)
Aspartate aminotransferase increased	6 (21)	0 (0)	6 (21)
Dysgeusia	5 (18)	0 (0)	5 (18)
Vomiting	5 (18)	0 (0)	5 (18)
Hoarseness	4 (14)	0 (0)	4 (14)
Sore throat	4 (14)	0 (0)	4 (14)
Dehydration	3 (11)	0 (0)	3 (11)

This table demonstrates the toxicities observed in the entire study population (n = 29) with a frequency greater than 10%.

TABLE 3. Treatment Efficacy<zaq;29><zaq;30>

Evaluable Patients	Value	
6-mo PFS, % (95% CI)[a]	32 (18-51)	
PFS, median (95% CI), d[a]	107.5 (72-164)	
PFS, median, mo	3.5	
6-mo OS, % (95% CI)[a]	71 (53-85)	
OS (KM estimate), median (95% CI), d[a]	301 (182-372)	
OS, median, mo	9.8	
Best overall response rate, No. (%)	42%<zaq;31>	
Progressive disease	10 (36)	
Stable disease	3 (11)	
Partial response	11 (39)	
Complete response	1 (3)	
Off treatment before 8-wk scan	3 (11)	
	Response Rate, % (No. of Responders/No. of Patients)	
Patients With Sequencing Results	Mutant	Wild Type
PI3K signaling pathway alterations	75 (6/8)	17 (2/12)
Noncutaneous squamous cell carcinoma	86 (6/7)[b]	12 (1/8)
Cutaneous squamous cell carcinoma	0 (0/1)	25 (1/4)[c]
KMT2C/D mutations	33 (2/6)	50 (6/12)
Noncutaneous squamous cell carcinoma	66 (2/3)[d]	50 (5/10)
Cutaneous squamous cell carcinoma	0 (0/3)	50 (1/2)[c]

Abbreviations: CI, confidence interval; KM, Kaplan-Meier; OS, overall survival; PFS, progression-free survival.

This table describes the efficacy and outcomes among evaluable patients and patients with sequencing results.

[a]The proportions for 6-month survival and the 95% CIs were estimated with the Wilson score interval method.<zaq;32>

[b]The remaining patient had stable disease as the best response to therapy.

[c]The patient had a KDR (VEFGR2) S110F mutation and exhibited a complete response.

[d]Both patients who exhibited a response had synchronous mutations in the PI3K signaling pathway.

This study demonstrates that treatment with axitinib improves survival in patients with heavily pretreated recurrent or metastatic head and neck squamous cell carcinoma, that alternative response criteria enable the identification of patients with atypical radiographic responses, and that patients with PI3K pathway alterations may derive exceptional benefit from therapy. Clinically, this study provides evidence for the evaluation of axitinib alone or in combination with immunotherapy in a genomic biomarker–selected population.

AQ1: Please check the tables for accuracy in typesetting.

AQ2: Please confirm or correct the names and degrees of the authors, the affiliations, and the correspondence footnote. Please also provide a middle initial for Brummel if possible.

AQ3: Please confirm or correct the sentence as edited.

AQ4: According to journal style, gene symbols (not the full names) should be italicized, whereas protein symbols should not be italicized. Please ensure that proper formatting is used for symbols throughout the article (no changes have been made).

AQ5: Please note that in accordance with journal style for abstracts, the first person (“we”) has been eliminated from the abstract.

AQ6: Please confirm or correct the lay summary as edited; note that bullets have been added in accordance with journal style.

AQ7: Please confirm or correct the sentence as edited (especially the insertion of “inhibitor”).

AQ8: Please confirm or correct “wrongly” (originally “abhorrently”).

AQ9: Please confirm or correct the sentence as edited (especially “complete blood count” and “thyroid-stimulating hormone”).

AQ10: Please verify the accuracy of the dosages.

AQ11: Please confirm or correct the sentence as edited (especially “complete blood count,” “comprehensive metabolic panel,” “thyroid-stimulating hormone,” and “urinalysis”).

AQ12: Please complete the unfinished sentence “Response to therapy” or delete it.

AQ13: Please confirm or correct “42.6% [n = 13]” (perhaps this should be “46% [n = 13]”).

AQ14: Please confirm or correct "12 patients (43%)" (Table 1 cites 11 patients with respect to a PD-1 inhibitor).
AQ15: Here and elsewhere, please confirm or correct "42%" if this is based on 12 patients (12/28 = 0.429; ie, 43%); please also confirm or correct "53%" if this is based on 15 patients (15/28 = 0.5357; ie, 54%).
AQ16: Please confirm or correct "3 of 8 [37%]" (perhaps this should be "3 of 8 [38%]" or "3 of 8 [37.5%]").
AQ17: Please confirm or correct the sentence as edited (especially "wild-type patients"); please also confirm or correct "39%" (2/12 = 0.167; ie, 17%).
AQ18: Please confirm or correct "VEGFR1, VEGFR2, and VEGFR3" as edited as well as "c-Kit" ("c-kit" is cited in the abstract).
AQ19: Please confirm or correct "response rate" (originally "RR").
AQ20: Please confirm or correct "patients with PD-L1 ≥ 1%" (perhaps this should be "patients with PD-L1 expression ≥ 1%" or something else).
AQ21: Please confirm or correct the sentence as edited (especially "refractory to PD-1 inhibitors").
AQ22: Please confirm or correct "its function on tumor cells or supporting cells" as edited; please also rewrite "to the pivotal for of VEGF/VEGFR signaling" in the last sentence of this paragraph for greater clarity.
AQ23: Please confirm or correct "noncutaneous squamous cell carcinoma" (originally "non-cSCC").
AQ24: Please confirm or correct "39.2%" (perhaps this should be "39.3%" [11/28 = 0.3929]).
AQ25: Please confirm or correct the funding support section, the conflict-of-interest section, and the author contributions as edited.
AQ26: Please confirm or correct the figure legend as edited.
AQ27: In Table 1, please confirm or correct "Age (n = 28), y" and "Previous exposure to PD-1 inhibitor (n = 11 [39.2%])" as edited. Please also confirm or correct the data (for example, perhaps "n = 11 [39.2%]" should be "n = 11 [39.3%]" [11/28 = 0.3929]).

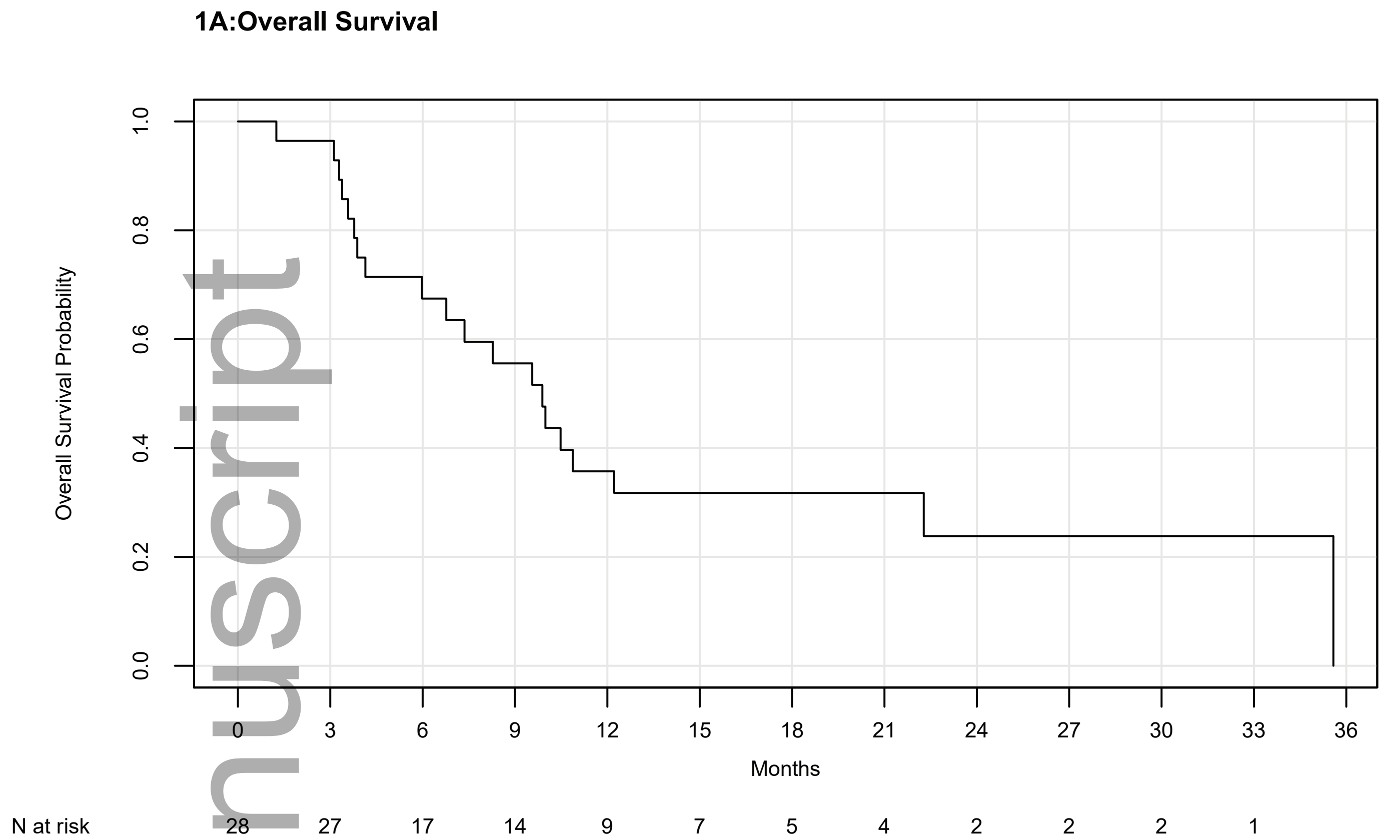
AQ28: According to the footnote, Table 2 concerns the entire study population of 29 patients, but the percentages seem to be based on 28 patients. If there is a discrepancy, please make any necessary changes.

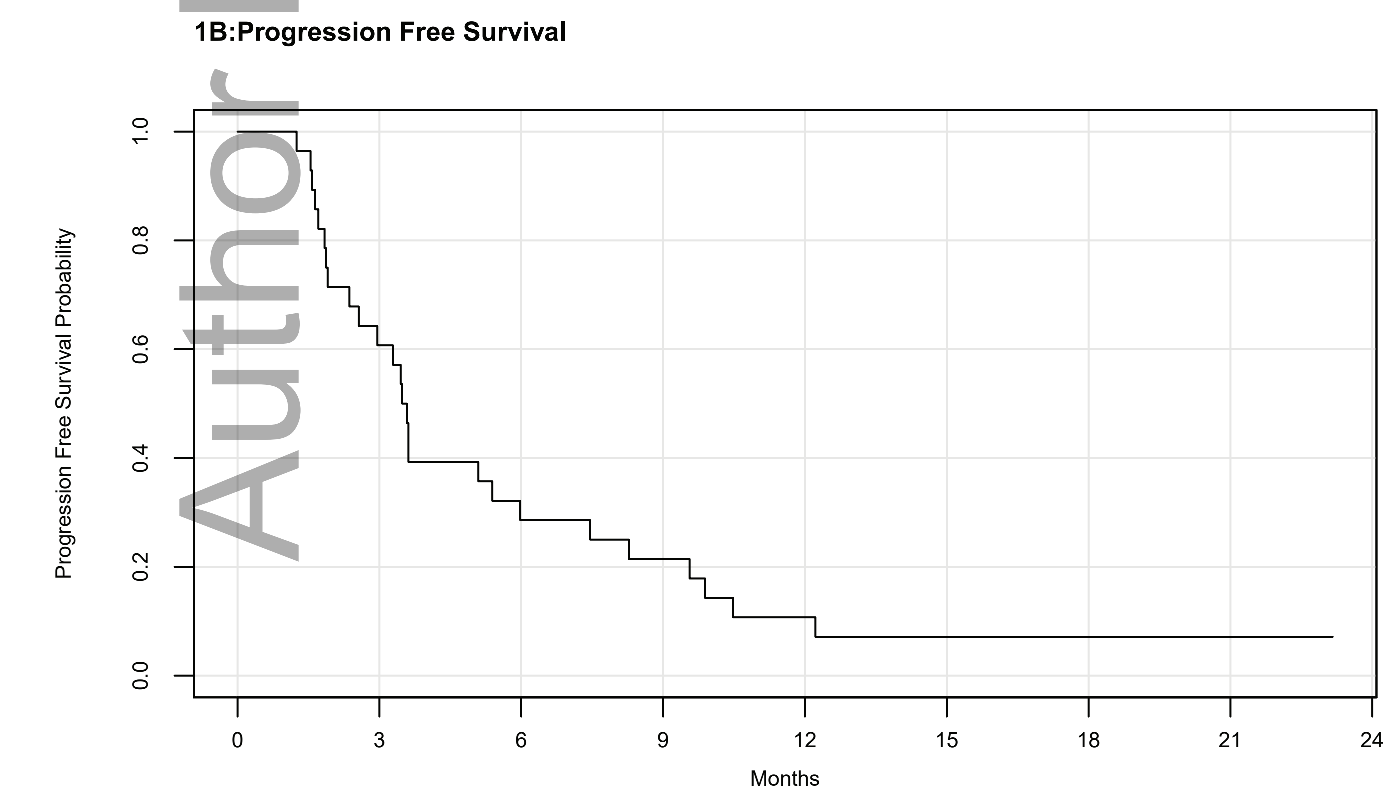
AQ29: In Table 3, please confirm or correct the data (for example, perhaps “1 (3)” should be “1 (4)” [1/28 = 0.036]).

AQ30: In Table 3, please confirm or correct the addition of “Evaluable Patients” and “Patients With Sequencing Results” (as replacements for “A” and “B”). Please also confirm or correct the addition of “PFS, median, mo” and “OS, median, mo” and the editing of “Response Rate, % (No. of Responders/No. of Patients).”

AQ31: In Table 3, please consider replacing “42%” with an n value and “42” in parentheses.

AQ32: In Table 3, please confirm or correct the footnotes and their citations as edited.





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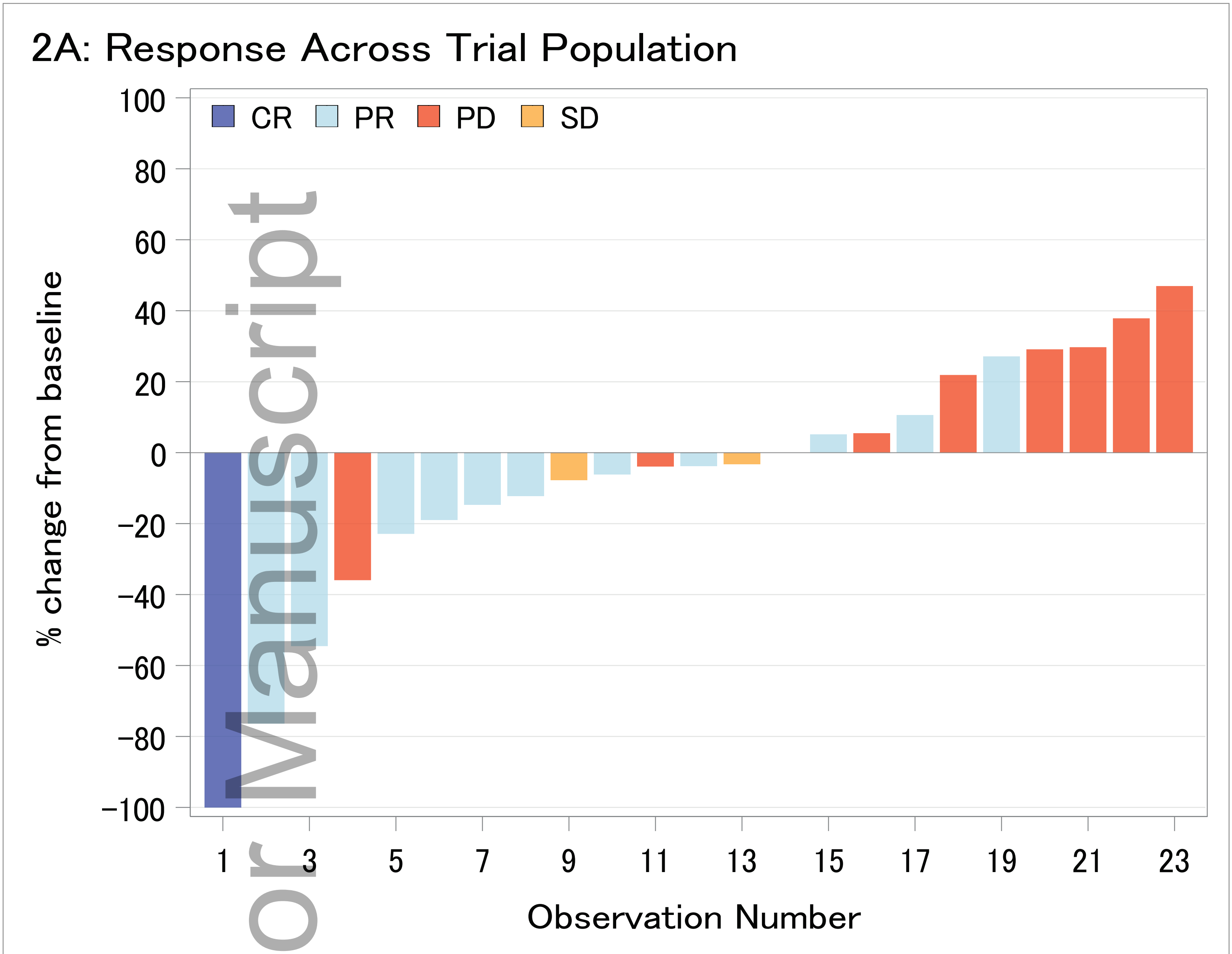

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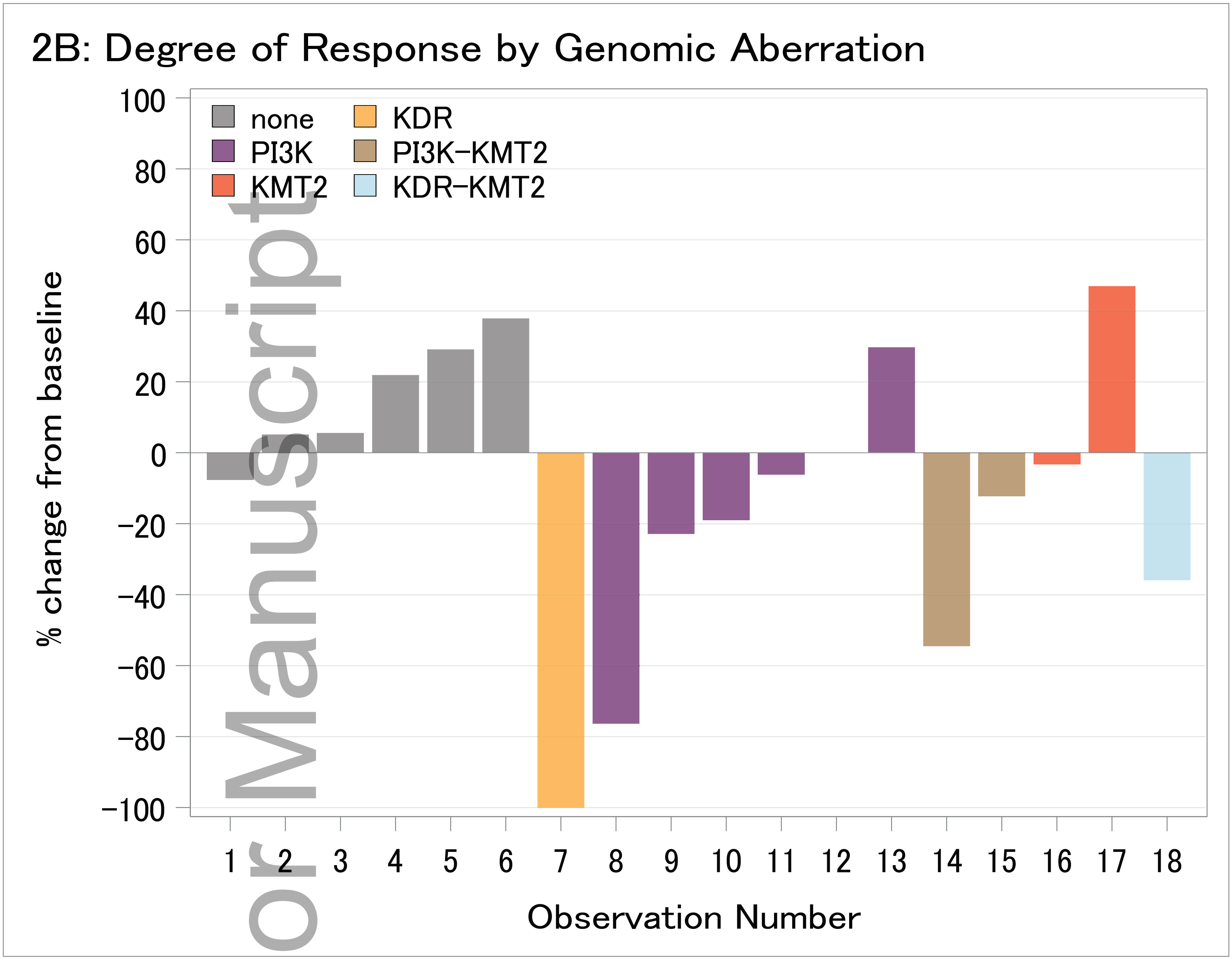

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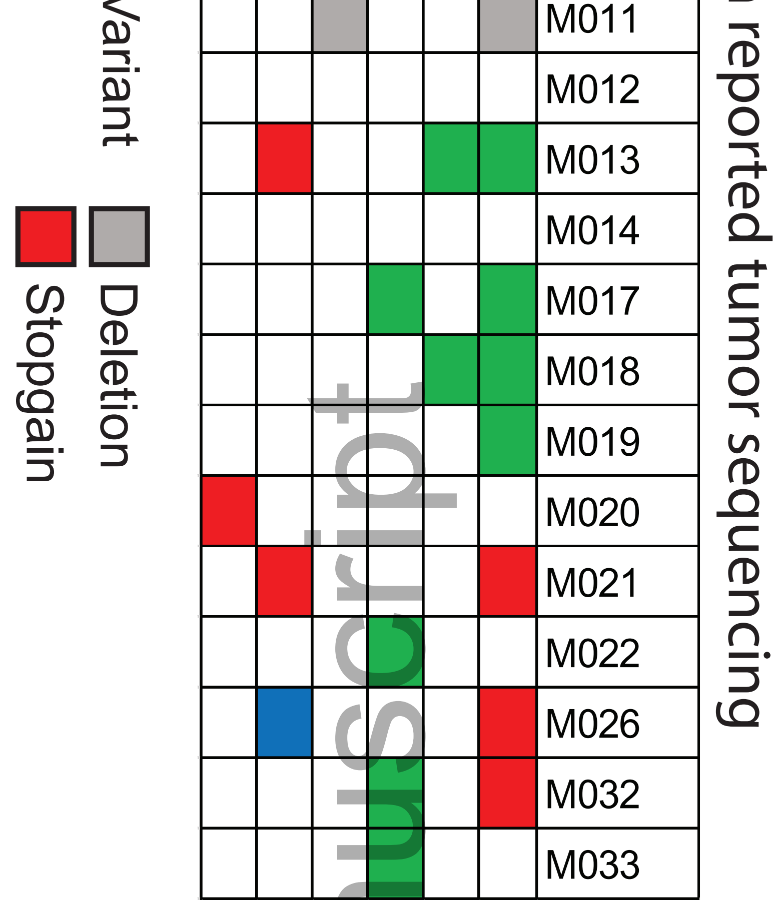

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