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Pharmacodynamic and therapeutic pilot studies of single-agent ribavirin in patients with human papillomavirus–related malignancies

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Abstract

Objectives: Ribavirin inhibits eukaryotic translation initiation factor 4E (eIF4E), thereby decreasing cap-dependent translation. In this two-part study, we assessed the pharmacodynamic effects and therapeutic potential of ribavirin in human papillomavirus (HPV)–related malignancies.

Methods: In the pharmacodynamic study, ribavirin (400 mg BID for 14 days) was evaluated in 8 patients with HPV-positive localized oropharyngeal carcinoma with phosphorylated-eIF4E (p-eIF4E) 30%. In the therapeutic study, ribavirin (1400 mg BID in 28-day cycles, continuously dosed) was evaluated in 12 patients with recurrent and/or metastatic HPV-related cancer. Dose interruptions or reductions were allowed according to prespecified criteria. Toxicities were assessed in accordance with National Cancer Institute Common Terminology Criteria for Adverse Events version 4; response was assessed using Response Evaluation Criteria in Solid Tumors version 1.1. Patients remained on study until disease progression or unacceptable toxicity.

Results: Six patients were evaluable in the pharmacodynamic study: 4 had decreased p-eIF4E after 14 days of ribavirin. In the therapeutic study, 12 patients were evaluable for toxicity, and 9 were evaluable for response. Among these, median follow-up was 3.5 months, and best overall

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Conflict of interest statement

Bharat Burman, Nora Katabi, and Richard Wong: none declared. Scott Drutman is an employee and stockholder at Volastra Therapeutics. Matthew Fury is an employee and stockholders at Regeneron Pharmaceuticals. Alan Ho: advisory board/consulting at Eisai Pharmaceuticals, Sanofi Genzyme (Aventis), Novartis, AstraZeneca, Bristol-Myers Squibb, Genentech/Roche, Sun Pharmaceuticals, Ayala Pharmaceuticals, Regeneron, TRM Oncology, CureVac, Kura Oncology, Exelixis, Rgenta, Inxmed, Prelude Therapeutics, Merck, Ignyta, and Klus Pharma; data safety and monitoring committee for Affymune. David Pfister: advisory board/consulting at Boehringer Ingelheim and Incyte; clinical trial support from Merck and Hookipa Pharma.

response was stable disease in 5 patients and progression of disease in 4 patients. Median progression-free survival was 1.8 months. The most common treatment-related adverse events (grade >2) were anemia, dyspnea, and hyperbilirubinemia. All patients had anemia (grades 1–3), with 33% having at least 1 dose reduction.

Conclusion: Oral ribavirin decreases p-eIF4E levels and is well-tolerated. However, a clear signal of efficacy in patients with recurrent and/or metastatic HPV-related cancers was not observed. (NCT02308241, NCT01268579)

Keywords

Ribavirin; human papillomavirus; HPV-related cancers; eIF4E; cap-dependent translation

INTRODUCTION

Human papillomavirus (HPV) is an oncovirus that has been implicated in the pathogenesis of nearly all cervical cancers and a substantial proportion of head and neck (HN), anal, penile, vaginal, and vulvar cancers. The number of HPV-related malignancies in the US remains above 30,000 per year, driven primarily by an increased incidence of HPV-related oropharyngeal squamous cell carcinomas (SCCs) despite decreased numbers of cervical cancers. While the prognosis of localized HPV-related cancers is generally favorable, recurrent and/or metastatic (R/M) disease carries a poor prognosis with a median overall survival of about 1 year [1–5]. Standard therapies developed for R/M HPV-related cancers improve clinical outcomes, but are not curative, leaving a persistent need to develop new therapies. For instance, the PD-1 checkpoint inhibitor pembrolizumab has a modest overall response rate of ~20% in R/M HNSCC and ~15% in advanced cervical cancer [5–7].

There is substantial interest in developing tissue agnostic therapies that target the biology of HPV-driven oncogenesis. HPV causes malignant transformation primarily through expression of E6 and E7 oncoproteins, which target p53 and pRb tumor suppressors for proteasomal degradation, respectively. E6 and E7 also cause the translation of mRNAs with 5' 7-methyl-guanosine caps [8–12]. Activation of “cap-dependent translation” promotes cancer growth and survival through upregulation of cap-dependent proto-oncogene transcripts such as CCND1, VEGF, BCL2, MMP9, and c-MYC, as well as E6 and E7 [13–16].

The central regulator of cap-dependent translation is the eukaryotic translation initiation factor 4E (eIF4E), which is regulated by oncogenic PI3K (phosphatidylinositol 3-kinase)/AKT/mTOR signaling [17]. Stimulated by growth factors, cellular stress, or changes in nutrients, PI3K/AKT activates the mTORC1 kinase complex, which in turn phosphorylates the translational repressor protein eIF4E-binding protein-1 (4E-BP1). Hyperphosphorylated 4E-BP1 releases eIF4E to interact with the translation initiation complex to initiate cap-dependent translation [8]. Approximately 80% of HNSCCs possess increased PI3K/AKT/mTOR signaling, and phosphorylated-eIF4E (p-eIF4E) is detected in almost all HPV-related HNSCCs [18, 19]. Elevated levels of eIF4E and cap-dependent translation are also seen in cervical cancer, a disease almost entirely driven by HPV [20]. Inhibition of eIF4E suppresses the growth of HNSCC and cervical cancer cell lines *in*

vitro[21, 22] and in mouse xenograft models[23], nominating eIF4E as a putative therapeutic target for HPV-related cancers.

Ribavirin is a guanosine ribonucleoside analog with activity against a wide range of viruses. Although its mechanism of action is not entirely understood[24], ribavirin mimics the 7-methyl-guanosine 5' cap of mRNA, thereby blocking eIF4E binding and inhibiting translation of capped viral transcripts [25–27]. Ribavirin targets eIF4E and suppresses tumor growth in hypopharyngeal cancer cells, chemo-resistant nasopharyngeal cell lines, and xenografts [27, 28]. In HPV-positive cervical cancer cell lines and xenografts, ribavirin induces cell cycle arrest and apoptosis [29]. Clinically, ribavirin has activity against laryngeal papillomatosis, a rare HPV-related disease of the upper airway [30, 31]. Ribavirin reduced eIF4E levels in patients with relapsed acute myelogenous leukemia (AML) [32, 33]. Taken together, these data suggest that ribavirin may possess activity in HPV-related malignancies by targeting eIF4E [34, 35].

Herein, we describe a two-part study of single-agent ribavirin. First, we conducted a 14-day pharmacodynamic study to evaluate if ribavirin can reduce p-eIF4E levels in patients with localized HPV-related HNSCC. We subsequently conducted a pilot therapeutic study of ribavirin in patients with R/M HPV-related malignancies.

PATIENTS AND METHODS

Both studies were single-institution phase I studies approved by the institutional review board at Memorial Sloan Kettering Cancer Center. All patients gave informed written consent.

Pharmacodynamic Study

Eligible patients had localized HNSCC and a biopsy of the primary tonsil and/or base of tongue or involved neck lymph nodes that showed SCC positive for p16 by immunohistochemistry, with or without HPV on *in situ* hybridization, and p-eIF4E 30% on immunohistochemistry. Other inclusion criteria were age 18–65; Karnofsky performance status 80; adequate hematologic, renal, and liver function; negative pregnancy test (if of childbearing potential); ability to swallow oral medications; and agreement to undergo biopsy after ribavirin treatment (if the patient did not undergo planned surgery). Exclusion criteria were prior chemotherapy or radiation for tonsil or base-of-tongue cancer, 10 pack-years of smoking, history of hemolytic anemia or thalassemia, active infection or other serious medical condition that would prevent protocol treatment, current or prior treatment with ribavirin, active hepatitis B or C infection, and clinically significant cardiovascular disease.

Ribavirin was self-administered at 400 mg PO BID (800-mg flat total daily dose) for 14 days (and no less than 10 days). The safety and efficacy of this dosing was delineated in hepatitis C clinical trials [36, 37]. Medication compliance was assessed by pill diaries and pill counts at each visit. Surgical resection or research biopsy was performed on Day 15 or at the discretion of the surgeon. Toxicities were assessed in accordance with National

Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4. All evaluable patients were able to be maintained on the 400-mg BID dose.

The primary objective of the study was to determine whether 2 weeks of ribavirin decreased tumor expression of p-eIF4E among patients with HPV-associated tonsil or base-of-tongue cancer. Formalin-fixed, paraffin-embedded tissue was processed using established methods. p-eIF4E staining was performed using standard methodology with a primary antibody against phospho-eIF4E-ser209 (Cell Signaling Technologies #9741). p-eIF4E was scored as the percentage of the total cells that stained positive. Positive p-eIF4E was defined as 30% of cells with cytoplasmic and/or nuclear staining [19, 21, 38, 39].

Therapeutic Study

Eligible patients had R/M HPV-associated SCC of the cervix, anus, vagina, vulva, penis, or oropharynx and had received at least 1 prior systemic therapy for R/M disease. HPV was confirmed by p16 immunohistochemistry, with or without HPV on *in situ* hybridization, except for cervical cancer, where SCC was presumed to be HPV related. Biopsies were not required, otherwise the other inclusion/exclusion criteria were similar to those for the pharmacodynamic study.

Ribavirin was self-administered at 1400 mg PO BID (2800-mg total daily dose) with food in 28-day cycles with continuous dosing. This dose was chosen because it was the recommended phase II dose (RP2D) based on the phase I trial of ribavirin in AML [33]. Medication compliance was assessed by pill diaries and pill counts at each visit. Dose interruptions or reductions were allowed according to prespecified criteria: dose level -1, 1000 mg PO BID; dose level -2, 800 mg PO BID; dose level -3, 600 mg PO BID. Toxicities were assessed according to CTCAE version 4. Cross-sectional imaging (CT or MRI) was obtained at baseline and after every 2 cycles. Response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Patients remained on study until disease progression or unacceptable toxicity.

The primary objective was to evaluate RECIST v1.1 response with ribavirin in patients with R/M HPV-related malignancies. Ribavirin would be considered worthy of further study if response was observed in at least 2 of 12 subjects, with the probability of missing an effect (observing 0–1 responses if the true response rate is 30%) of 0.09. This cutoff for response rate was based on historical efficacy data of other drugs used in second-line or greater for R/M head and neck, cervical, and anal cancers, for which response rates are typically 15%–20% [2–5]. Secondary objectives were to (1) determine overall survival and progression-free survival, (2) determine the safety and tolerability of ribavirin in this patient population, and (3) perform tissue correlative studies for the purpose of hypothesis generation.

RESULTS

Pharmacodynamic Study

From April 2011 to September 2013, 8 patients were enrolled, of whom 6 were evaluable (Figure 1). One withdrew consent, and 1 had no viable cells in the posttreatment biopsy

specimen for analysis. Of these 6 patients, 4 had a marked decreased in p-eIF4E staining after 14 days of ribavirin (Table 1, Figure 2). We took these results as evidence that oral ribavirin could reach its target and exert an effect on p-eIF4E in the tumors and deemed the drug worthy of further evaluation in our therapeutic pilot study.

No grade >2 toxicities were observed during the study. Anemia, the most common toxicity associated with ribavirin, was not observed in any patient; therefore, no dose reductions or interruptions were required for anemia. The most common laboratory abnormalities possibly related to treatment were a grade 1 increase in alanine aminotransferase in 3 patients and a grade 1 increase in bilirubin in 1 patient.

Of the 6 patients, 5 had paired pre- and post-treatment imaging available. Although clinical response was not formally addressed in the pharmacodynamic study, all 5 patients appeared to have stable disease. One patient had a decrease in FDG avidity on PET scan at the primary site (standardized uptake value, from 13.2 to 4.3).

Therapeutic Study

From January 2015 to April 2017, 12 patients were enrolled in this study (Figure 3). Median age was 59 years (range, 47–69). Five patients had anal canal cancer, 3 had cervical cancer, and 4 had oropharyngeal cancer. Patients had received a median of 3 lines of prior chemotherapy (range, 1–6), including chemotherapy given concurrently with radiation, and 11 had received prior radiation therapy. Baseline characteristics are presented in Supplementary Table 1.

All patients were evaluable for toxicity. The most common toxicity attributed to ribavirin was anemia: 25% grade 1, 50% grade 2, and 25% grade 3. One patient required discontinuation of ribavirin and removal from the study due to refractory grade 3 anemia that persisted after 2 dose reductions. One episode of grade 3 hyperbilirubinemia was attributed to the drug, which resolved after holding the drug and dose reduction. In total, 7 patients required dose reductions (4 for anemia and 3 for abnormal liver function tests), with 3 reaching dose level –1 (1000 mg BID) and 4 reaching dose level –2 (800 mg BID). Among the 12 evaluable patients, the median number of cycles completed was 2 (range, 1–8), and the median number of days of treatment was 34 (range, 19–222). There were no grade 4 or 5 toxicities in the study. No other grade 3 toxicities could clearly be attributed to the study drug. Treatment-related toxicities that occurred in 2 patients are listed in the Table 2.

Nine patients were evaluable for response. One patient with anal cancer withdrew consent after 1 cycle owing to rectal bleeding believed to be due to clinical progression, without radiographic evidence of progression. Another patient with anal cancer withdrew consent after 1 cycle after imaging showed tumor growth. One patient with oropharynx cancer withdrew consent after 1 cycle owing to overall clinical decline. At the time of analysis, all patients had been removed from the study drug, with a median follow-up of 3.5 months (range, 1.4–10.4). Among these 9 patients, the best overall response was stable disease in 5 patients and progression of disease in 4 patients (Table 3). None of the patients had radiographic response that met the criteria for partial response (the largest RECIST response

was -6%); thus, we did not meet our prespecified cutoff of 2 responses to warrant further study of ribavirin for this indication. Median progression-free survival was 1.8 months. At the time of analysis, 8 patients had died, and 4 had been censored, with a median survival of 8.6 months (95% confidence interval, 4.3–19.4) (Figure 4).

All patients came off study within the first 2 cycles (8 for progression of disease, 1 for toxicity), except for 2 patients: 1 with oropharynx cancer that progressed after 8 cycles (222 days) and 1 with anal cancer that progressed after 6 cycles (173 days), although the clinical benefit of ribavirin in these cases is difficult to ascertain. The patient with stable disease for 222 days appeared to have an indolent course leading up to the trial; this patient had been treated with 5 lines of therapy over the course of 2 years for metastatic head and neck cancer and most recently had stable disease on single-agent paclitaxel for >7 months. The patient with stable disease for 173 days was treated relatively early in the course of his metastatic anal cancer, and thus the overall trajectory of his disease was not clear.

Nine of the 12 patients had molecular testing performed on their tumor (MSK-IMPACT; paired normal and tumor DNA). Typical for HPV-driven cancers, 5 tumors had mutations in PIK3CA (3 with E545K and 2 with E542K), and 1 had mutations in PIK3R1 (R755Fs), suggesting that response to ribavirin was not associated with tumors driven by activating mutations in the PI3K pathway (Table 3).

DISCUSSION

This two-part study demonstrated that oral ribavirin reduces p-eIF4E in early-stage HPV-related HNSCCs; however, it is unclear whether single-agent ribavirin suppresses tumor growth or leads to improved clinical outcomes in patients with R/M HPV-related cancers. Intriguingly, a ribavirin dose as low as 800 mg/day reduced tumor levels of p-eIF4E, indicating on-target effects. We chose a higher dose of 2800 mg/day for our therapeutic study based on the RP2D from a phase I trial of ribavirin in AML [33]. Although we observed no adverse events grade >3, all patients had some degree of anemia, with grade 3 anemia occurring in 25% of patients. Anemia, a known toxicity of ribavirin, caused 33% of patients to require at least 1 dose reduction, including 1 patient who developed refractory anemia and withdrew from the study. Of note, the AML study may have reached a higher RP2D because the protocol was more tolerant of anemia and ribavirin was combined with cytarabine, which can reduce its plasma levels [33].

The pharmacodynamic window study builds on our recently published report that HPV-positive oropharynx cancers universally express p-eIF4E, as well as on preclinical studies demonstrating that HPV E6 increases eIF4E-mediated cap-dependent translation through PI3K/AKT/mTOR signaling. Consistent with other studies, our data confirms that ribavirin significantly decreases p-eIF4E and cap-mediated translation. However, there are some limitations to our pharmacodynamic study. It is possible that the levels of p-eIF4E in tumors is inherently dynamic and an untreated control group was not analyzed to verify the biomarker decrease was specifically related to ribavirin treatment. Notably, the 2 patients without reduction had stable p-eIF4E staining between the two biopsies. Although 1 patient

did have a decrease in PET signal of the primary tumor during the window study, the short treatment interval was not adequate to evaluate for significant clinical responses.

In our therapeutic pilot study, significant clinical activity was not detected. Two patients had atypically long intervals of stable disease (173 and 222 days), though the contribution of ribavirin to this clinical outcome is unclear. Limitations of this therapeutic evaluation were the small sample size and toxicities of high-dose ribavirin. The lack of activity may reflect that targeting eIF4E alone is insufficient to induce major tumor regressions. Alternatively, primary and acquired resistance to ribavirin can be mediated by increased expression of sonic hedgehog transcription factor glioma-associated protein 1 (GLI1), which drives UDP-glucuronosyltransferase-dependent glucuronidation of ribavirin [32, 33, 40, 41]. In phase I studies of ribavirin in AML, elevated GLI1 expression at baseline was associated with a lack of initial response, and in patients who did have an initial response, elevation in GLI1 was associated with acquired resistance [33, 40]. Interestingly, the hedgehog pathway inhibitor vismodegib restored sensitivity to ribavirin *in vitro* by downregulating GLI1[40], suggesting a combination of ribavirin and vismodegib could have therapeutic potential.

Beyond ribavirin, new potent and selective agents targeting eIF4E or cap-dependent translation are being developed for clinical testing and they may offer a better therapeutic window to elicit efficacy against HPV-related cancers [42]. Preclinical data demonstrate that inhibition of eIF4A, another subunit of the translation initiation complex, downregulates proteins associated with AKT and MAPK signaling; a phase I/II trial of a small molecule inhibitor is underway [43]. In addition, inhibitors of MAPK interacting kinase 1/2, such as tomivosertib, which blocks eIF4E phosphorylation, represent an area of active investigation with multiple compounds in clinical development [44]. However, all of these are still in early development, and studies in HPV-related diseases are needed.

Targeting the PI3K/AKT/mTOR pathway and downstream effectors remains an attractive therapeutic strategy for the treatment of HPV-positive and HPV-negative solid-tumor malignancies that rely on hyperactivation of this pathway. In our therapeutic study, 5 of 9 tumors with genomic profiling had activating PI3K mutations. To overcome feedback-based resistance mechanisms to traditional mTOR inhibitors, recent efforts to target the pathway have focused on targeting activating mutations of PI3K isoforms [45]. A phase I basket study of taselisib, a selective inhibitor of class I PI3K α , γ , and δ isoforms, showed an acceptable safety profile and a clinical response rate of 8.9% in 146 patients [46]. Interestingly, higher response rates were seen in patients with head and neck cancers (19.0%) and cervical cancer (10.5%) who may have HPV-positive tumors (subgroup analysis pending). However, in a subgroup analysis of buparlisib (BKM120), another oral pan-PI3K inhibitor, plus paclitaxel in patients with platinum-pretreated HNSCCs, objective response rate was slightly lower in patients with HPV-positive tumors (35.3%) than in patients with HPV-negative tumors (39.6%), although this difference was not statistically significant [47]. A trial of buparlisib combined with cisplatin and radiation therapy is underway and enrolling patients with HPV-positive and HPV-negative HNSCCs (NCT02113878). Whether these selective PI3K inhibitors inhibit eIF4E and cap-dependent translation remains to be determined.

Combination therapies using ribavirin and other agents with nonoverlapping toxicity profiles may lead to durable responses by increasing potency and overcoming resistance mechanisms. We recently reported the results of a phase Ib trial of induction chemotherapy with afatinib, ribavirin, and weekly carboplatin and paclitaxel for stage IVA/IVB HPV-positive oropharyngeal SCC in which patients received ribavirin daily for 3 21-day cycles before definitive treatment [48]. The best overall response was unconfirmed partial response in 6 patients (67%), stable disease in 2 patients (22%), and progression of disease in 1 patient (11%). Importantly, the regimen was safe and well tolerated, with no grade 3 anemia events. The utility of combining ribavirin with immune checkpoint inhibitors remains unclear. Two recent animal model studies revealed that inhibiting cap-dependent translation by targeting either eIF4F or MAPK interacting kinase 1/2 decreases expression of PD-L1, leading to tumor regressions and CD8+ T cell activation [49, 50]. However, increased eIF4F activation was associated with response to anti-PD-1 in patient melanoma samples [50]. In HPV-related cancers, targeting cap-dependent translation may decrease expression of E6 and E7, a source of unique viral antigens that is increasingly targeted by emerging cellular therapies and vaccines. Therefore, studies that combine cap-mediated translation inhibitors and immunotherapy for R/M HPV-positive cancers should be carefully considered.

CONCLUSION

In conclusion, ribavirin reduces levels of p-eIF4E, a downstream effector of PI3K/AKT/mTOR signaling and central regulator of cap-dependent translation. Although single-agent ribavirin downregulates a critical biomarker linked to HPV oncogenesis, it may be insufficient to control tumor growth in patients with R/M HPV-related cancers. More potent and selective inhibitors of PI3K and eIF4 isoforms, or combinations of ribavirin and other agents, may overcome resistance mechanisms and are currently being studied in clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

4E-BP1	eIF4E-binding protein-1
AML	acute myelogenous leukemia
CTCAE	Common Terminology Criteria for Adverse Events

eIF4E	eukaryotic translation initiation factor 4E
GLI1	glioma-associated protein 1
HN	head and neck
HPV	human papillomavirus
p-eIF4E	phospho-eIF4E
POD	progression of disease
RECIST	Response Evaluation Criteria in Solid Tumors
R/M	recurrent and/or metastatic
RP2D	recommended phase II dose
SCCs	squamous cell carcinomas
SD	stable disease

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Highlights

- Therapies targeting the viral etiology of HPV-related cancers are lacking
- HPV E6 and E7 oncogenesis is dependent on eIF4E-mediated cap-dependent translation
- Ribavirin effectively reduces eIF4E levels in patients with HPV-related cancers
- Ribavirin monotherapy likely has limited clinical efficacy in HPV-related cancers
- Rational combinations with ribavirin or other eIF4E inhibitors should be considered

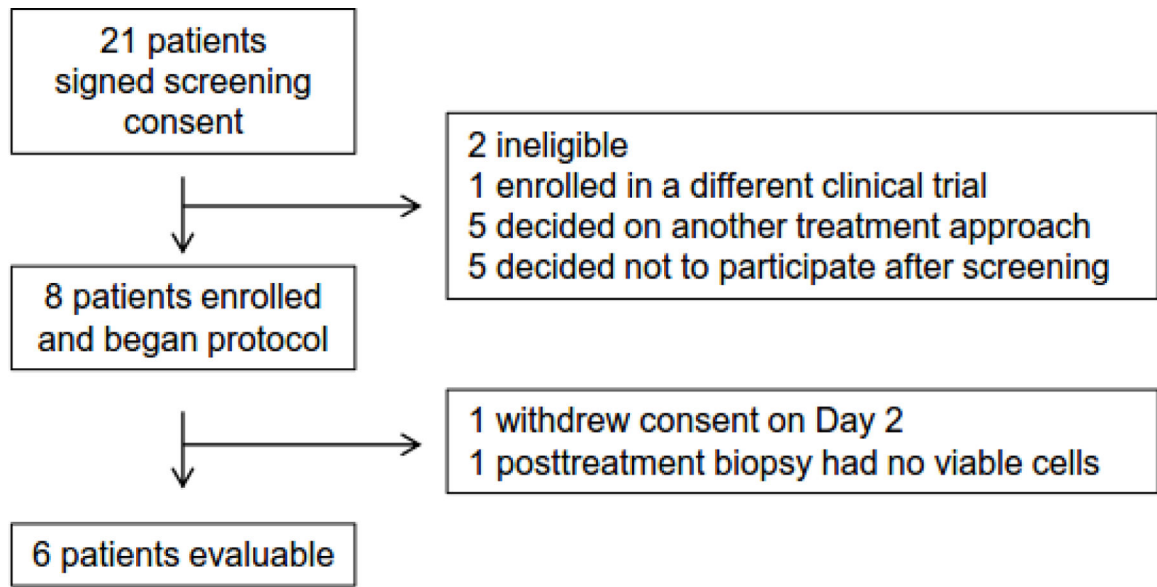


Figure 1.
Enrollment schema of the pharmacodynamic study.

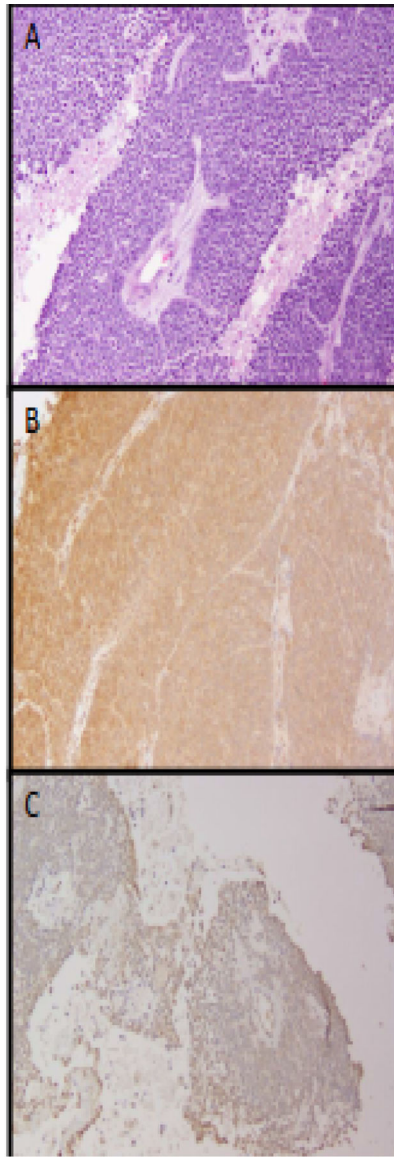


Figure 2. Reduction in phospho-eIF4E staining by immunohistochemistry in Patient 2. (A) Pretreatment biopsy specimen showing characteristics of nonkeratinizing squamous cell carcinoma. (B) Pretreatment biopsy specimen stained with phospho-eIF4E. (C) Posttreatment biopsy specimen stained with phospho-eIF4E.

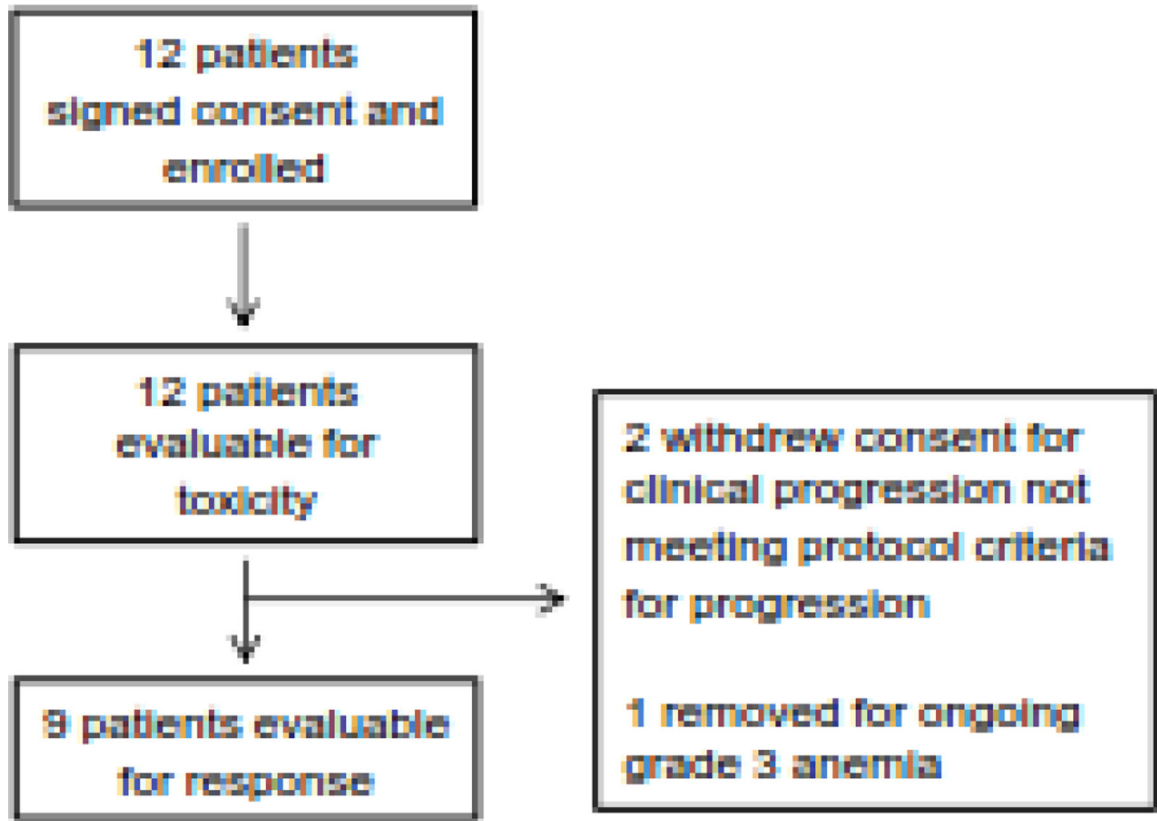


Figure 3.
Enrollment schema of the therapeutic study.

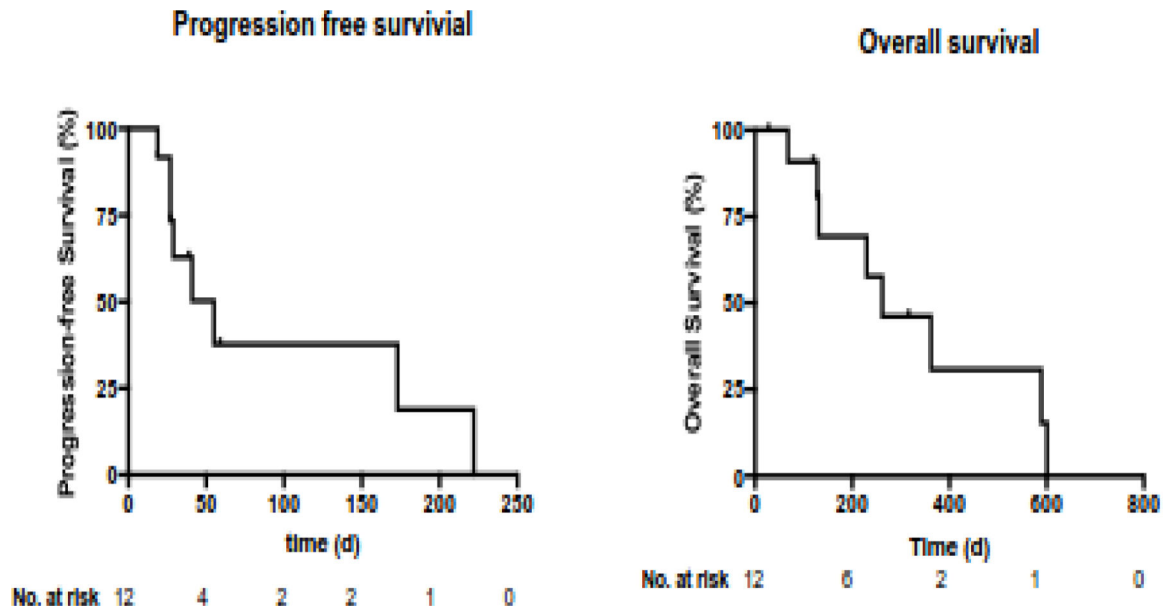


Figure 4. Progression-free survival and overall survival curves for patients in the therapeutic study.

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Table 1.

Summary of phospho-eIF4E staining in the 6 evaluable patients in the pharmacodynamic study

Patient	Baseline phospho-eIF4E, %	Posttreatment phospho-eIF4E, %
1	70	30
2	90	30
3	70–80	40–50
4	40	40
5	80	50
6	80	80

Results are scored as the percent of tumor cells that had positive nuclear and/or cytoplasmic phospho-eIF4E staining by immunohistochemistry. Samples from subjects 3 and 4 were surgical specimens; the remainder were fine-needle aspirates

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Table 2.

Treatment-related toxicities occurring in 2 patients in therapeutic study

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4 or 5	Any grade
Anemia	2 (17)	3 (25)	1 (8)		6 (50)
Dyspnea	4 (33)		1 (8)		4 (42)
Blood bilirubin increase	1 (8)	1 (8)	1 (8)		3 (25)
Fatigue	1 (8)	2 (17)			3 (25)
Anorexia	2 (17)				2 (17)
Aspartate aminotransferase increase	2 (17)				2 (17)
Fever	1 (8)	1 (8)			2 (17)
Gastroesophageal reflux	2 (17)				2 (17)
Weight loss	2 (17)				2 (17)
Alanine aminotransferase increase	1 (8)				2 (17)
Alkaline phosphatase increase	1 (8)				2 (17)

Data are no. (%).

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Table 3

Summary of patient characteristics, best overall response, duration of response, reason for discontinuation, and molecular testing among patients in the therapeutic study.

Patient	Tumor type	Prior lines of chemotherapy	Best overall response	Best response by RECIST, %	Treatment duration, days	Reason for discontinuing study	Molecular testing
1	Oropharynx	5	SD	-6	222	Disease progression	ASXL1; DNMT3B; ATR; EP300; MLL3; PIK3R1
2	Anal	1	SD	6	59	Withdrawal of consent ^a	Not performed
3	Cervix	3	SD	8	39	Toxicity (anemia)	PIK3CA; SOX2; EIF4A2; TP63; BLM; FBXW7; FGFR1; GRIN2A; MGA; MLL2; NUP93; PAK7; RAD51C; RPTOR; SMO; SUFU; TSC2
4	Anal	2	POD	39	27	Disease progression	TP63; CTNNB1
5	Anal	2	SD	-1	55	Progression (nontarget lesion)	PIK3CA; B2M; BRAF; CRKL; EP300; ERG; KDM6A; LATS2; MLL3; PIK3C3; RYBP; STAG2; TET1
6 ^b	Oropharynx	3	-	-	27	Clinical decline (pain)	ERBB2; TP63; DICER1; MDC1; NOTCH1; ATR
7 ^b	Anal	6	SD	1	27	Withdrawal of consent ^a	PIK3CA
8	Anal	2	SD	3	173	Disease progression	PIK3CA, GNA11; TGFBR2; ANKRD11; DICER1; DIS3; SPEN; KDM6A
9	Cervix	3	POD	0	19	Progression (nontarget lesion)	Not performed
10	Oropharynx	4	POD	5	29	Disease progression	GSK3B
11 ^b	Oropharynx	5	-	-	19	Withdrawal of consent ^a	PIK3CA; FBXW7; FOXA1; PTEN
12	Cervix	2	POD	-1	41	Progression (nontarget lesion)	Not performed

POD, progression of disease; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

^aReasons for consent withdrawal: Patient 2: rectal bleeding; Patient 7: radiographic progression not meeting RECIST criteria; Patient 11: clinical decline.

^bInevaluable for response: Patients 6 and 11: no interval imaging was performed; Patient 7: radiographic progression not meeting RECIST criteria, incomplete cycle.