A Pilot Study to Assess the Pharmacodynamic Effects of Ribavirin in Patients with Tonsil and/or Base of Tongue Squamous Cell Carcinoma

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program

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Table of Contents

1.1 PROTOCOL SUMMARY AND/OR SCHEMA

1.2 Summary

This will be a single-institution, non-randomized, pilot study for 7 patients at MSKCC Main Campus.

We wish to study ribavirin in this patient population because: (1) eIF4E can function as an oncogene; (2) we have recently found that expression of phosphorylated eIF4E is associated with p16-positive tonsillar squamous cell carcinoma (3) ribavirin has been shown to decrease eIF4E levels *in vivo.*

The Primary Aim of this pilot study is to explore if ribavirin therapy for 2 weeks decreases tumor expression of phosphorylated eIF4E among patients with tonsillar and/or base of tongue squamous cell carcinoma.

Subjects will have tonsil and/or base of tongue squamous cell carcinoma that is previously untreated.

Subjects will not have a history of more than 10 pack-years of tobacco use. Subjects will have had a prior diagnostic surgical or core needle biopsy. Patients for whom definitive local-regional treatment (primary surgery or primary radiation therapy +/- chemotherapy, given sequentially or concurrently) is planned will be offered participation in the study. Subjects will take ribavirin 800 mg/day in divided doses for two weeks, which is a standard time frame for pre-operative planning. Patients will be evaluated in clinic by the medical oncologist during week 1 and week 2. (For patients who undergo research biopsy, this will be performed in the clinics of the Head and Neck Disease Management Team.)

Patients will be considered done with treatment at time of surgery and/or research biopsy.

Post-surgical (or post research biopsy) management will be off protocol, according to established standards of care.

1.3 Schema

New Diagnosis Tonsil and/or Base of Tongue SCC

❒ 10 unstained slides must be available from the initial diagnostic biopsy

❒ Written Informed Consent

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Treatment

❒ Ribavirin 800 mg/day, in 2 divided doses, for approximately 14 days
❒ Day 1, Day8: H&P, CBC, comp panel

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Approximately Day 15

❒ Surgery or Research Biopsy at MSKCC
❒ Discontinue Ribavirin

↓

Off-Study

☑ Patients are off-study as of Day of Surgery (or Research Biopsy)
☑ Post-operative (or post Research Biopsy) management is off protocol, per standards of care

2.1 OBJECTIVES AND SCIENTIFIC AIMS

2.2 Primary:

2.1.1

To explore if ribavirin therapy for 2 weeks prior to tumor resection decreases tumor expression of phosphorylated eIF4E among patients with tonsillar and/or base of tongue squamous cell carcinomas that express phosphorylated eIF4E.

2.3 Secondary:

2.2.1

In pre- and post-treatment tumor samples, to explore the pharmacodynamic effects of ribavirin on molecules that may be regulated, directly or indirectly, by eIF4E (eg, p16, p21, EGFR, p53).

2.2.2

In pre- and post-treatment tumor samples, to explore if ribavirin reduces the expression of HPV-16 oncoproteins E6 and E7

3.1 BACKGROUND AND RATIONALE

3.2 HPV/p16-positive HNSCC is a distinct clinical and biological entity

HNSCC comprises at least two distinct clinical entities: HPV-positive tumors and HPV-negative tumors (1). Approximately half of oropharynx (tonsil, base of tongue) squamous cell carcinomas are HPV-positive, but tumors arising at other sites in the upper aerodigestive tract (i.e., oral cavity, hypopharynx, or larynx) generally are not associated with HPV (1). HPV-negative tumors typically occur in individuals with histories of tobacco abuse, whereas HPV-positive HNSCC usually is not associated with tobacco abuse (2, 3). In a prospective randomized clinical trial of cisplatin plus concurrent radiation in HNSCC, HPV-positive squamous cell carcinomas arising in the oropharynx were associated with superior overall survival compared with HPV-negative oropharynx tumors (4).

Potentially, differences in the molecular pathogenesis mechanisms of these tumors account for their distinct treatment responsiveness. Landmark cytogenetic studies of HNSCC demonstrate frequent gene loss at the locus encoding p16 and p14 (*CDKN2A*), and amplification at the gene locus encoding cyclin D1 (5-7) Whereas p16 loss and cyclin D1 amplification characterize HPV-negative (tobacco-related) tumors, p16 overexpression and downregulation cyclin D1 typify HPV-positive tumors (8). The high p16/low cyclin D1 profile in HPV-positive tumors is due to loss of negative feedback on p16 when the E7 viral oncoprotein inactivates the pRB tumor suppressor protein (1, 9). Immunohistochemical detection of p16 is a marker for HPV-associated carcinogenesis in HNSCC (10-12).

In summary, HPV-positive and HPV-negative head and neck cancers are distinct at the molecular pathology level. There are converse molecular profiles for HPV-positive tumors

(ie, overexpression of p14 and p16; downregulation of cyclin D1; p53 wildtype) and HPV-negative tumors (ie, overexpression of cyclin D1; downregulation p14, p16 and p21; p53 mutant) (3, 8, 10, 13-15), and these molecular profiles can inform the development of clinical studies.

3.3 Rationale for targeting eIF4E in HPV/p16-positive tumors

The translation initiation factor eIF4E is a central regulator of gene expression (16). eIF4E binds the 7-methyl guanosine (m^7G) cap structure in the 5' end of mRNAs, and selectively stimulates the translation of several oncogenic transcripts that are repressed under normal circumstances. Malignancy related mRNAs that are regulated at the level of translation by eIF4E include cyclin D1, VEGF, bcl-2, MMP-9 and Pim-1 (16, 17). Wendel and colleagues demonstrated that eIF4E functions as an oncogene and confers resistance to therapeutic mTOR inhibition in a murine lymphoma model (18, 19).

In 1999 clinicopathologic study of 65 HNSCC surgical cases, Nathan and colleagues reported that expression of eIF4E was detectable by immunohistochemistry in all of the tumors. Expression of eIF4E in histologically tumor-free margins was present in 55% of specimens, and was associated with a significantly increased risk of tumor recurrence (20).
This group subsequently demonstrated that antisense inhibition of eIF4E mRNA reduced the malignant properties of a HNSCC cell line (FaDu), as measured by increased contact inhibition, reduced growth in soft agar, and reduced tumor growth in xenograft mouse models (21). Growth inhibitory effects also were observed when another HNSCC cell ine (UMSCC22B) was subjected to eIF4E silencing with siRNAs (22). As further evidence of eIF4E's central role in translation regulation in HNSCC cells, eIF4E-targeted suicide gene therapy in a murine model of HNSCC (SCC-7 cells) improved disease-free survival (23).

Our current interest in eIF4E in head and neck cancer is an offshoot of our DMT's research program into inhibitors of the PI3K/Akt/mTOR pathway (IRB 06-129, IRB 08-138, IRB 09-028, IRB 09-131). To explore if expression levels of any proteins in this pathway are associated with p16 status, we constructed a tissue microarray (TMA) with 46 archived tonsil squamous cell carcinoma specimens (24). The TMA was interrogated with antibodies directed against proteins in this pathway and against p16. The antibody panel included anti-$eIF4E^{Ser209}$, because phosphorylation of eIF4E appears to be essential for oncogenic activity (19, 25).

We demonstrated that downstream components of the PI3K pathway, such as phosphorylated Akt and S6, are expressed in the majority of the specimens (24), in agreement with the results of the Head and Neck Cancer Tissue Array initiative (26). Sixty-five percent of the tumors expressed p16, the marker for HPV-driven carcinogenesis (10-12). The salient new finding was that p16 expression was associated with expression of phosphorylated eIF4E (p= 0.03, Table 1) (24). Expression of p16 also correlated with expression of p21 (p = 0,02, Table 1) (24), in agreement with the immunohistochemistry study of Hafkamp (8). There was no association between expression of p16 and phosphorylated S6 or phosphorylated 4E-BP1. In agreement with multiple reports regarding HPV-status and tobacco history (2, 3), we found a significant correlation between history of tobacco abuse (>10 pack/years) and absence of p16 expression (p = 0.01) (24).

Table 1. Associations between anti-p16 and 8 other antibodies (24)

Antigen	Number p16 positive	Number p16 negative	P value*
p21			0.02
Pos (%)	25 (78)	7 (22)	
Neg (%)	5 (38)	8 (62)	
PTEN			0.02
Pos (%)	24 (77)	7 (23)	
Neg (%)	6 (40)	9 (60)	
Phospho eIF4E^{Ser209}			0.03
Pos (%)	26 (74)	9 (26)	
Neg (%)	4 (9)	7 (91)	
Phospho 4E-BP1$^{Thr37/46}$			1.0
Pos (%)	20 (67)	10 (33)	
Neg (%)	9 (64)	5 (36)	
Phospho AKT^{Ser473}			0.12
Pos (%)	21 (75)	7 (25)	
Neg (%)	9 (50)	9 (50)	
Phospho S6$^{Ser240/244}$			1.0
Pos (%)	19 (63)	11 (37)	
Neg (%)	10 (67)	5 (33)	
Phospho S6$^{Ser235/236}$			0.75
Pos (%)	13 (65)	7 (35)	
Neg (%)	17 (71)	7 (29)	
p53			1.0
Pos (%)	11 (65)	6 (35)	
Neg (%)	19 (68)	9 (32)	

*P values determined by Fisher's exact test

Note: For the following antibodies, tumor sample was missing from the TMA for one patient each: p21, p53, phosphorylated S6$^{Ser240/240.}$ Tumor sample was missing from the TMA for two patients each for: phosphorylated 4E-BP1$^{Thr37/46}$ and phosphorylated S6$^{Ser235/236}$ (24).

The association between p16 and phosphorylated eIF4E expression has potential implications for clinical study. In the context of the central role of eIF4E in the translation of proteins encoded by capped mRNAs (27), the association between p16 and phosphorylated eIF4E may reflect interplay between virus and cellular translational apparatus. In cells infected with herpes simplex virus-1 or vaccinia virus, phosphorylation of eIF4E by Mnk-1 is critical for viral protein synthesis and replication (28, 29). It is not known if similar mechanism occurs in HPV-positive HNSCC, but HPV is associated with increased cap-dependent translation in differentiating cervical cancer cell lines (30), and eIF4E staining is increased in cervical neoplasia (31).

In summary, several observations indicate that eIF4E is an attractive research target in HPV/p16 positive tonsillar squamous cell carcinoma: (1) eIF4E expression has prognostic significance in HNSCC (2) eIF4E knockdown in HNSCC cell lines is associated with reduced tumorigenicity (3) phosphorylated eIF4E is associated with p16 status in a tonsil squamous cell carcinoma tissue microarray (4) eIF4E is critical for viral protein synthesis in other systems.

3.4 Ribavirin is a broad-spectrum antiviral agent that inhibits eIF4E

In 1972, the guanosine ribonucleotide analogue ribavirin (Virazole®; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was reported to have broad spectrum activity against both RNA and DNA viruses. The combination of ribavirin + peginterferon (PEG-IFN) α-2b is an effective treatment for chronic hepatitis C. The most common adverse events associated with ribavirin are mild anemia, cough, and rash. For patients treated with prolonged ribavirin, anemia usually is mild and hemoglobin remains in the normal range (32, 33). Risk factors for moderate to severe anemia with ribavirin are advanced age, low body weight, hepatic or renal insufficiency, and baseline marrow dysfunction (34-36). In the current study, eligibility criteria exclude patients with factors that might put them at increased risk of anemia with ribavirin.

The pivotal trial (n = 1530 subjects) establishing PEG-IFN α-2b + ribavirin efficacy against chronic hepatitis C showed that treatment for 48 weeks with PEG-IFN α-2b (1.5 µg/kg/week) and a flat dose of ribavirin (800 mg/day) was statistically superior to IFN α-2b or low-dose (0.5 µg/kg/week) of PEG-IFN α-2b and weight-based dose of ribavirin (1000 – 1200 mg/day) (37). A subsequent randomized clinical trial (n = 5027 subjects) demonstrated that a weight-based ribavirin (800 – 1400 mg/day) + PEG-INF α-2b was more effective than flat-dose ribavirin (800 mg/day) + PEG-IFN α-2b (38). However, the improvement in sustained virologic response was modest (44.2% for weight-based dosing versus 40.5% for flat dosing), and appeared to be restricted to patients with HCV genotype 1. Additionally, mean hemoglobin levels decreased to a lesser extent in the flat-dose group than in the weight based-group (38). In the current protocol, ribavirin will be given at flat dose of 800 mg/day to optimize patient safety.

Ribavirin has been reported to have clinical activity against laryngeal papillomatosis (LP) in a small non-randomized pilot study (39). LP, a rare condition with an incidence of approximately 7.1 per million in the U.S., is an HPV-related disease in which the upper airway becomes progressively occluded with mucosal papillomas. Repeated laser surgery for removal of lesions has been commonly applied as a management strategy. In the pilot study, patients (3 adults, 1 child) received pre-operative ribavirin (23 mg/kg daily) on the day of laser surgery, and continued oral ribavirin at the same daily dose for an additional 6 months (only 3 months for the child). The viral strain was HPV-6 in the papillomas of three patients, and was HPV-11 in one patient.

Treatment was generally well tolerated, with only one patient requiring a 25% dose reduction for anemia, which resolved with continued treatment at the reduced dose. Ribavirin treatment was associated a decrease in observable disease on follow up examinations, as determined by a previously described mapping score. Ribavirin treatment was associated with an increase in the time interval between successive required surgeries by a factor of at least 2 in all patients (39). No pharmacodynamic correlates were done in this study. The efficacy of ribavirin against laryngeal papillomatosis was confirmed in a subsequent case report (40), and ribavirin is considered to be one treatment option for this uncommon entity (41).

The mechanism of ribavirin's antiviral activity is not conclusively established, and at least five potential mechanisms have been proposed (42). For example, ribavirin is a potent competitive inhibitor of cellular inosine monophosphate dehydrogenase (IMPDH), thereby depleting GTP pools that are necessary for viral nucleic acid synthesis. Ribavirin also inhibits viral 7-methylguanosine (m^7G) RNA cap synthesis in vaccinia virus (43, 44). These two mechanisms may be complementary: by reducing levels of competing GTP, ribavirin

may be more effective as a capping inhibitor (42). The precise mechanism of ribavirin may be influenced by the particular virus and cell type, but the capping inhibition mechanism may be potentially relevant for those viruses that produce capped transcripts, such as HPV-16.

In 2004, Borden and colleagues proposed that ribavirin competitively prevents eIF4E from binding the m^7G cap of mRNAs, thereby inhibiting translation of proteins encoded by capped mRNAs (45). Because many capped mRNAs are important for oncogenesis, these findings suggested that ribavirin might potentially have activity against eIF4E-overexpressing cancers. However, this particular mechanism has been called into question as other labs hav e not been able to reproduce the finding that ribavirin mimics the m^7G cap (46, 47), perhaps due to assay conditions (48). While the precise mechanisms of ribavirin require further study, subsequent clinical study in AML patients demonstrates that ribavirin decreases eIF4E levels *in vivo* (49).

The *in vivo* demonstration of the impact of ribavirin on eIF4E levels was also reported by Borden and colleagues. They evaluated ribavirin in a proof-of-principle study for patients with relapsed or refractory AML, a disease which commonly overexpresses eIF4E (49). The starting dose of ribavirin was 1000 mg/day, with dose increases allowed for lack of response at 14 days. Treatment was well tolerated; no patient required dose reduction for toxicity. Among 13 enrolled patients, there was 1 complete response, 2 partial remissions, 2 blast responses, 4 stable diseases, 2 progressive diseases, and 2 patients were inevaluable (49).

Ribavirin treatment decreased levels of eIF4E mRNA (Table 2) and protein (Table 2, Figure 1) in AML patients (49) (including Supplemental Data). Prior to treatment, all patients evaluated had elevated eIF4E mRNA levels versus healthy controls. With a single exception, patients receiving 28+ days of therapy had a 2- to 10-fold drop in eIF4E RNA levels by day 28 (Table 2), and these levels remained reduced for the duration of treatment. Levels of eIF4E protein and other pathway proteins were assessed by Western blot in several patients, demonstrating decreases in eIF4E levels with ribavirin treatment in each case (Figure 1) (49). Based on these encouraging studies, a follow-up clinical trial is underway in AML (www.clinicaltrials.gov; NCT01056523). Ribavirin is also being evaluated in advanced breast cancer (www.clinicaltrials.gov; NCT01055757), due to evidence of aberrant eIF4E activity in that disease (50, 51).

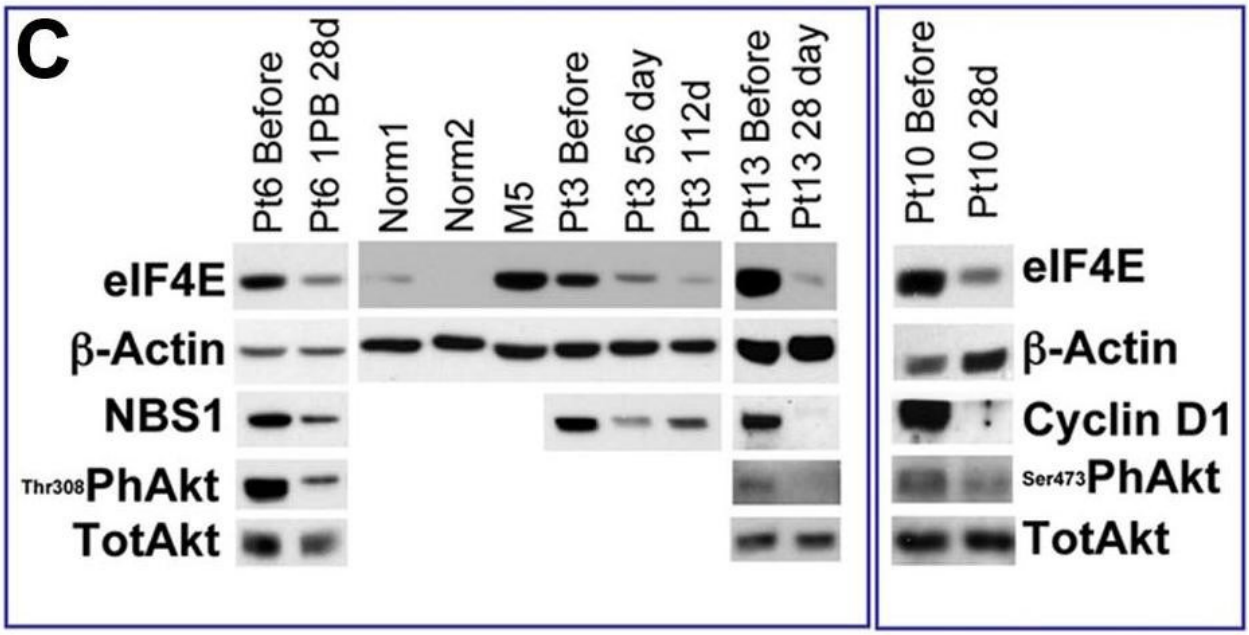
Table 2: eIF4E levels in AML before and after ribavirin treatment (49).

Sample	eIF4E mRNA Baseline[a]	eIF4EmRNA Day 28[b]
Pt1	3.00 ± 0.99	0.50 ± 0.25
Pt3	6.33 ± 0.82	0.32 ± 0.32
Pt6	6.40 ± 0.88	0.64 ± 0.10
Pt9	2.99 ± 0.44	1.66 ± 0.32
Pt10	7.91 ± 1.04	0.24 ± 0.09
Pt12[c]	5.06 ± 0.68	1.10 ± 0.15
Pt13	3.34 ± 0.79	0.53 ± 0.12
	eIF4E Protein Baseline	**eIF4E Protein Day 28**
Pt 7[d]	6.38± 0.17	0.67 + 0.01[e]
Pt 8[d]	4.03± 0.28	N/A
Pt 11[d]	4.36±0.18	0.26±0.04

Note: RNA levels were quantified using quantitative real time PCR and normalized to multiple housekeeping genes to ensure that there were no errors due to unexpected changes in the normaliser genes. RNA levels are presented ± standard deviations.

[a] eIF4E RNA levels before treatment relative to normal controls
[b] eIF4E mRNA levels during treatment divided by baseline values
[c] Patient 12 was treated for only 19 days.
[d] For patients 7, 8, and 11, there was no RNA available for analysis. Thus, analysis of the intensity of eIF4E immunostaining in micrographs was monitored. Analysis was done measuring pixel intensities over the same surface area of micrographs using Adobe Photoshop CS4 for the skin biopsy (patient 7) or bone marrow biopsy (patient 8) or by measuring average pixel intensity per cell for confocal micrographs (patient 11).
[e] For patient 7, eIF4E protein level was assessed in skin biopsy on day 100 of treatment (49).

Figure 1: eIF4E activity and levels are reduced by ribavirin treatment in AML. Western blot analysis was carried out using the antibodies indicated (49)



The demonstration of efficacy in AML motivates the exploration of ribavirin in other malignancies. Head and neck cancer is an obvious choice due to clinical and preclinical data demonstrating the eIF4E appears to play a significant role (20, 21, 52). Additionally, ribavirin has potent antitumor activity against a HNSCC cell line (FaDu) that overexpresses eIF4E. *In vitro*, ribavirin inhibits phosphorylation of Akt and reduces anchorage independent growth of FaDu cells (53). In a xenograft model using FaDu cells, 20 days of treatment with ribavirin yielded a 6-fold reduction in mean tumor volume in treated animals, compared with the control group (45). The hypothesis that this growth inhibition may be mediated by ribavirin's effect of eIF4E is supported by the observation that reduction of eIF4E expression in FaDu cells with antisense RNA also reduces the tumorigenic and angiogenic properties of FaDu cells (21). Taken together, we feel that these observations support a pilot study of ribavirin in head and neck cancer. In order to enrich for patient population that expresses the eIF4E target, the study is limited to never smokers (or past oligo smokers) with tonsillar squamous cell cancer (see Table 1 above).

3.5 Rationale for Tissue Pharmacodynamic Studies

To obtain proof-of-principle regarding the hypothesis that ribavirin is an inhibitor of eIF4E (45, 48, 49), we will compare pre and post treatment tumor levels of phosphorylated eIF4E by immunohistochemistry. Expression of phosphorylated eIF4E is associated with p16 positive tonsil squamous cell carcinoma (24) [see Table 1 herein]. In AML, ribavirin therapy results in decreased levels of eIF4E mRNA and protein (49) [see Figure 1 and Table 2 herein]. eIF4E can function as an oncogene (18), and pharmacodynamic demonstration of downregulation of eIF4E would provide a mechanistic basis for further development of ribavirin in HPV-positive head and neck cancer.

As a secondary endpoint, we will explore if ribavirin alters levels of selected proteins that are regulated by eIF4E at the translational level. In a model system with primary human mammary epithelial cells (HMECs), eIF4E activates p16 and p21 and represses EGF receptor (17). This eIF4E-induced pattern of expression (high p16, high p21, and low EGFR) seen in HMECs (17) is very similar to the protein expression patterns seen in HPV-positive HNSCC (3, 8, 24). The striking similarities between expression patterns induced by eIF4E in a HMEC model system and the expression patterns in HPV-positive HNSCC prompt exploratory pharmacodynamic assays involving pre and post-treatment tissue in this study. We wish to obtain pilot data to determine if ribavirin impacts expression of p16, p21, and/or EGFR in patient tumors in this study, which could strengthen the hypothesis that ribavirin is an inhibitor eIF4E activity.

We also wish to explore if ribavirin treatment effects the expression of viral oncoproteins E6 and E7. HPV-16 uses a single promoter to drive expression of a bicistronic mRNA that encodes E6 and E7. Synthesis of both E6 and E7 from the bicistronic transcript is dependent on an m^7G cap structure on the 5'end of the mRNA (54). In view of the hypothesis that ribavirin may interfere with translation of cap-dependent mRNA transcripts (43-45), we wish to obtain pilot data regarding whether inhibition of production of E6 and E7 might be a mechanism of action for ribavirin in HPV-positive tonsil squamous cell carcinoma.

3.6 Summary and Future directions

In this study, up to 7 evaluable subjects will be treated with ribavirin for 14 days to evaluate the pharmacodynamic effects of ribavirin in HPV-positive oropharynx SCC. We feel that 14 days is adequate to demonstrate the pharmacodynamic effects of ribavirin because (1) Ribavirin pharmacology is characterized by rapid absorption and rapid distribution (Section 5.1); (2) Ribavirin often causes mild anemia within 2 weeks, consistent with rapid onset clinical activity of the drug (61); (3) For other targeted therapies with well-defined mechanisms of action in oncology, pharmacodynamic activity is evident in 15 days or less (62, 63); (4) In the pilot study of ribavirin in AML, the first response assessment occurred at 14 days (49).

Squamous tumors of tonsil and base of tongue, both oropharynx subsites, share the same underlying molecular pathology in which HPV figures prominently in the development of malignancy (1). Primary surgical resection or primary radiation +/- chemotherapy (concurrent or sequential) may be offered to patients with either tonsil or base of tongue SCC. Because HPV-driven pathology is a shared characteristic of SCC at both of these subsites, we feel that it is appropriate to include both tonsil and base of tongue SCC in this study.

If the pilot data from this study is consistent with the hypothesis that ribavirin is an inhibitor or eIF4E, we would pursue further clinical studies of ribavirin in head and neck cancer. In view of the reported supra-additive effects of cisplatin + eIF4E inhibition against HNSCC cancer cell lines (22), we would consider a phase I/II study of ribavirin + low dose weekly cisplatin + external beam radiation therapy for patients with stage III/IVB HPV-positive HNSCC. The low-dose cisplatin regimen would likely be better tolerated than the current standard high-dose cisplatin regimens (64, 65). We would also wish to consider a phase I/II study of ribavirin + cisplatin + docetaxel as induction chemotherapy for patients with stage III/IVB HPV-positive HNSCC. The use of 2 cytotoxic agents in this experimental induction regimen would likely be better tolerated than the current standard induction chemotherapy in HNSCC which is three cytotoxic agents (cisplatin, docetaxel, 5-fluorouracil) (66). Another study option might be developed for patients who undergo primary surgery for head and neck cancer. In view of the report that expression of eIF4E in histologically tumor free margins is associated with increased risk of recurrence (20), a post-operative treatment regimen incorporating ribavirin would be an attractive study concept. Each of the study concepts outlined above would provide the opportunity to obtain adequate tumor tissue for correlative studies to further analyze the mechanistic effects of ribavirin. Finally, in view of the worldwide burden of cervical carcinoma which is also characterized by elevated eIF4E expression (31), these findings potentially could have implications beyond head and neck cancer clinical research.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This will be a single institution non-randomized study for patients with tonsil and/or base of tongue squamous cell cancer. This is a pilot study to obtain pharmacodynamic data regarding the effects of ribavirin on tonsil and/or base of tongue squamous cell cancer. After patients provide informed consent, eIF4E expression in tumors will be measured by immunohistochemistry. Those patients with tumors that express eIF4E ≥ 30% will be deemed eligible for the study and will self-administer ribavirin for approximately 14 days. The primary endpoint is to explore if ribavirin decreases tumor expression of phosphorylated eIF4E among patients with tonsil and/or base of tongue squamous cell carcinoma. Immunohistochemistry will be performed on pre-treatment pathology samples (e.g. diagnostic biopsy), as well as on the post-treatment pathology samples (e.g. definitive surgery or research biopsy, to describe the effects of ribavirin treatment on the expression of phosphorylated eIF4E.

4.3 Intervention

Patients will self-administer ribavirin for approximately 14 days prior to surgery. Patients will undergo safety assessments with clinic visits and routine laboratory studies at least once per week. On approximately day 15, patients will undergo planned surgical resection or research biopsy. Patients will be considered done with treatment at time of surgery or research biopsy. Post-operative (or post research biopsy) management will be according to standards of care off protocol.

For those patients who undergo research biopsy, this will be performed in the clinics of the Head and Neck Disease Management Team. Research biopsy will be performed on easily accessible lesions. The phrase “easily accessible tumor” designates tumors that may be biopsied in an office setting with minimal risk of complication and discomfort to the patient. Common techniques for office biopsy include fine needle aspirate of malignant neck node, or forceps cut biopsy of primary tumor (with local anesthetic). The method used for research biopsy in the clinic is at the discretion of the investigator.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

5.2 Ribavirin

Availability and Administration:
Generic ribavirin is commercially available as 200 mg, 400mg, and 600 mg tablets, which will be orally administered by the patients themselves. Ribavirin will be purchased from the hospital pharmacy. Tablets are supplied in bottles of 84 capsules (200 mg each). Ribavirin is available as blue-colored (shade depending on strength), capsule-shaped, film-coated tablets. Medication labels will comply with US legal requirements and be printed in English. They will supply no clinical information about the patient on the labels. The storage conditions for study drug will be described on the medication label. Ribavirin tablet may be taken with food or on an empty stomach.

Toxicities (> 10%):

Hematologic: anemia, thrombocytopenia, leukopenia, lymphopenia
Gastrointestinal: anorexia, nausea, diarrhea
Hepatic: elevated liver function tests
Pulmonary: dyspnea, cough
Reproductive: may be teratogen
Constitutional: fatigue, musculoskeletal pain
Dermatologic: alopecia, pruritis, rash
Neurologic: headache, dizziness
Endocrine and metabolic: hyperuricemia

Pharmacology:

Ribavirin oral tablet administration is characterized by rapid absorption with a t_{max} of approximately 2 hours, followed by rapid distribution and long terminal phases. Mean γ-phase half-life has been reported to be in the range of approximately 30 - 61 hours after a single dose, and 274 - 296 hours following multiple doses. Mean bioavailability is approximately 30 - 50%. Apparent plasma clearance is approximately 20 L/h. Population based pharmacokinetic models have identified factors associated with lower apparent clearance of ribavirin (female gender, higher age, and low body weight), but taken together these factors account for less than 30% of the variability in clearance (34, 67-69).

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

1. Prior diagnostic surgical or core needle biopsy, with confirmation of tonsil and/or base of tongue squamous cell carcinoma that is positive for expression of p16 and phosphorylated eIF4E, as determined by the Department of Pathology at MSKCC. The biopsy may be either of the tonsil, base of tongue, and/or an involved neck node. 2 unstained slides and/or tissue block must be available from the initial diagnostic biopsy

   Positive expression p16 and phosphorylated eIF4E is defined as >=30% of tumor cells with cytological and/or nuclear staining

2. Age ≥ 18 and ≤ 65 years of age

3. Karnofsky Performance Status ≥ 80

4.

Adequate organ function, as follows:

Adequate bone marrow reserve: absolute neutrophil count (ANC) ≥ 1.5 X 10^9/L, platelets ≥ 160 X 10^9/L, hemoglobin ≥ 12 g/dL

Hepatic: total bilirubin within 1.5 X upper limit of normal (ULN) ; alkaline phosphatase (AP), aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 2.5 X ULN

(Patients with Gilbert's syndrome as the cause of hyperbilirubinemia may be eligible if total bilirubin ≤ 2.5 X UNL)

Renal: Serum creatinine ≤ 1.3 mg/dL. Patients with serum creatinine > 1.3 mg/dL may be eligible if creatinine clearance (CrCl) ≥ 55 mL/min based on the standard Cockroft and Gault formula.

5. Patients of childbearing potential must have a negative serum pregnancy test within 14 days of treatment. Patients must agree to use a reliable method of birth control during and for 6 months following the last dose of study drug.

6. Ability to swallow oral medication.

7. Non-surgical patients: If primary radiation +/- chemotherapy (concurrent or sequential) is planned, patients must agree to undergo research biopsy after completion of ribavirin treatment.

6.2 Subject Exclusion Criteria

1. Prior chemotherapy or radiation for tonsillar or base of tongue squamous cell cancer
2. More than 10 pack-years of tobacco use
3. History of hemolytic anemia or thalassemia
4. Active infection or serious underlying medical condition that would impair the patient's ability to receive protocol treatment.
5. Current therapeutic anticoagulation with Coumadin (warfarin)
6. Current or prior treatment with ribavirin
7. Known active Hepatitis B or C
8. Any prior documented history of transient ischemic attack (TIA) or cerebrovascular accident (CVA)
9. New York Heart Association (NYHA) Grade II or greater congestive heart failure
10. Clinically significant peripheral vascular disease
11. History of unstable angina or myocardial infarction (MI) within the last 3 years

7.0 RECRUITMENT PLAN

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team at Memorial Sloan-Kettering Cancer Center (MSKCC).

Patient recruitment will occur in medical oncology clinics of the Head and Neck Disease management team at main campus. If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/research staff of the study.

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8.1 PRETREATMENT EVALUATION

All pre-treatment evaluations should be within 21 days prior to therapy, unless otherwise specified in this Section.

- Complete medical history including current medications, physical examination including evaluation of Karnofsky performance status.
- Pathology review at MSKCC must confirm diagnosis of tonsil and/or base of tongue squamous cancer that is positive for expression of phosphorylated eIF4E, at any time prior to treatment. Diagnostic biopsy may be of tonsil and/or involved neck node. 2 unstained slides and/or tissue block must be available from the diagnostic biopsy
- The following laboratory studies will be obtained within 21 days prior to therapy: Complete blood count with white blood cell differential and platelet counts; Comprehensive profile (including electrolytes, bicarbonate, blood urea nitrogen (BUN), creatinine, glucose, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, total protein, albumin, and glucose); prothrombin time and activated partial thromboplastin time
- Serum pregnancy test for women of childbearing potential within 14 days prior to therapy.
- Electrocardiogram within 8 weeks prior to therapy
- Baseline cross-sectional imaging of the neck (CT neck or MR neck) within 8 weeks prior to therapy

9.1 TREATMENT/INTERVENTION PLAN

9.2 Clinical Plan

The clinical intervention in this study is ribavirin therapy for approximately 14 days. Ribavirin 800 mg/day is administered in divided doses, 400 mg PO qAM and 400 mg PO qPM. Details regarding evaluations during treatment are provided in Section 10.

9.3 Immunohistochemistry (IHC)

Each formalin-fixed and paraffin-embedded tissue specimen will be processed for IHC, consistent with established methods in our group (70). The time from tumor removal at surgery to formalin fixation should be 2 hours or less.

The avidin-biotin immunoperoxidase technique will be employed. Antigen retrieval with citric acid (0.01%) for 15 minutes under microwave treatment commonly precedes incubation with the primary antibody (usually overnight at 4°C). Primary antibodies to be used include anti-phospho eIF4E^{Ser209} (rabbit polyclonal, Cell Signaling #9741), anti-p16 (mouse IgG, clone E6H4, Dako #K5334), anti-p21 (mouse IgG, clone EA10, Calbiochem #OP64), anti-EGFR (mouse IgG, clone 31G7, Ventana #28-005), anti-HPV16 E6 (mouse IgG, clone C1P5, Santa Cruz #sc-460), and anti-E7 (mouse IgG, clone 8C9, Invitrogen #28-0006). Diaminobenzidine will be utilized as the final chromogen and hematoxylin will provide the nuclear counterstain.

If limited tumor tissue is available, the top priority immunostain will be phosphorylated eIF4E.

If sufficient tissue is available, exploratory analysis of additional biomarkers may be performed. For example, if immunohistochemistry does not provide definitive data regarding levels of E6, E7, and p53, expression may be explored with RT PCR using established methods (71, 72).

Technical difficulty or inability to obtain results with certain antibodies or samples will not be considered a protocol violation.

10.1 EVALUATION DURING TREATMENT/INTERVENTION

10.2 Description of Evaluations

Patients will be provided with a pill diary at the start of treatment (Appendix A), and will be required to bring a updated pill diary to each clinic visit. If patients miss any doses of ribavirin, this will not be considered a protocol violation but the investigator retains the discretion to remove patients from study for non-compliance.

Every attempt will be made for patients to have clinic visits and laboratory tests on the indicated dates. However, dates may need to be altered for medical or personal or logistical reasons. Clinic visits and laboratory studies may occur 3 days before or 3 days after the scheduled date, if need be. Additional clinical visits and laboratory studies may be scheduled if clinically necessary, per investigator discretion.

- On Day 1, routine laboratory studies are obtained: complete blood cell count and comprehensive metabolic panel. Patients undergo history and physical.

- Days 1 – 14, approximately: ribavirin 800 mg/day in divided doses, 400 mg q AM and 400 mg q PM. Typically, this will be administered as two 200 mg tablets qAM, and two 200 mg tablets qPM. Use of one 400 mg tablet (instead of two 200 mg tablets) is also allowed, at the discretion of the investigator.

- On Day 8, Routine laboratory studies are obtained: complete blood cell count and comprehensive metabolic panel. Patients undergo history and physical.

- Day 15 (approximately): Surgical plan is resection or research biopsy of the tonsil and/or base of tongue squamous cell carcinoma, possibly with neck dissection at the discretion of the surgeon. Patients will be considered done with treatment at time of surgery or research biopsy. As such, the surgical or biopsy procedure is considered off-protocol, and the surgery or research biopsy will be performed at the discretion of the investigator. The protocol provides no guidance nor places any restrictions regarding surgical management.

  While every attempt will be made to schedule surgery or research biopsy on Day 15, the exact timing of surgery or research biopsy will be at the discretion of the surgeon. No ribavirin will be given after surgery or research biopsy.

- Post-operative (or post research biopsy) management will be per standard of care, off protocol.

10.3 Calendar of Events

	Baseline	Day 1[a]	Day 8	Day 14	Day 15
History and Physical	X	X	X		
CBC, comp panel	X	X	X		
PT/aPTT	X				
EKG	X				
CT or MRI of neck	X				
Ribavirin		800 mg PO daily X 14 days			
Surgery or research biopsy					X
Adverse Events (AEs)		Continuous Monitoring for AEs			

[a] This table shows approximate dates. Please see sections 9 and 10 for allowed variations in the timing of interventions and assessments

11.1 TOXICITIES/SIDE EFFECTS

This section provides guidelines for the clinical management of adverse events. The toxicities of ribavirin are presented in Section 5, and are listed again here:

Hematologic: anemia, thrombocytopenia, leukopenia, lymphopenia
Gastrointestinal: anorexia, nausea, diarrhea
Hepatic: elevated liver function tests
Pulmonary: dyspnea, cough
Reproductive: may be teratogen
Constitutional: fatigue, musculoskeletal pain
Dermatologic: alopecia, pruritis, rash
Neurologic: headache, dizziness
Endocrine and metabolic: hyperuricemia

Reporting requirements for adverse events are presented in Section 17.2.

All toxicities will be assessed according to CTC version 4. Dose reductions or drug stoppage are only required for those adverse events that the investigator feels are possibly, probably, or definitely related to ribavirin.

Subjects who take < 10 days of ribavirin prior to surgery will be considered inevaluable and will be replaced.

Any patient who requires dose reduction below 600 mg/day will be removed from study.

11.2 Hematologic Toxicity

The most common toxicity requiring dose reduction for ribavirin is anemia.

For any patient who experiences a fall in hemoglobin of > 2 g/dL (from Day 1 value), ribavirin dose will be decreased to 600 mg/day (200 mg qAM, 400 mg qPM). Any patients who develop a hemoglobin level of < 10 g/dL will be removed from study.

Any patient who develops a platelet count of < 100 K/mcL will be removed from study. Any patient who develops an absolute neutrophil count of <1.0 K/mcL will be removed from study. Dose delays and dose reductions are not required for uncomplicated leukopenia or uncomplicated lymphopenia.

11.3 Non-Hematologic Toxicity

For any patient who develops grade 2 toxicity (felt to be definitely, probably, or possibly related to ribavirin), the investigator may either (a) reduce the dose of ribavirin to 600 mg/day, or (2) remove the patient from study. Any patient who develops toxicity of grade 3 or greater will be removed from study.

Even when the patient meets criteria for continued treatment at full dose per the protocol, the investigator retains the discretion to delay treatment and/or reduce dose (to 600

mg/day) if there are safety concerns and/or logistical issues. Any dose reductions not specified in the protocol should be discussed with the principal investigator.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary objective of this study is based on immunohistochemistry (IHC), not a therapeutic or radiologic outcome. For details regarding IHC methods, see Section 9.2. As a secondary endpoint, we wish to explore the feasibility of using pre and post treatment FDG-PET/CT scans to assess the metabolic effect of ribavirin in tonsillar and/or base of tongue squamous cell carcinoma.

The volume(s) of interest (VOI) from the MRI neck (or CT neck) images will be matched to the FDG PET/CT images. Matched VOIs will be analyzed by visual and semiquantitative analysis, using the attenuation-corrected PET emission images. For semiquantitative analysis, VOIs are placed over the areas of focal FDG uptake in the neck nodal metastases. The intensity of FDG uptake in these regions is measured using the standardized uptake value (SUV), normalized to body height. The maximum pixel SUV was recorded. The imaging data initially available in units of microcuries per milliliter per voxel are decay corrected to the time of injection and converted into SUV units (73).

PET images will be reconstructed from the initial data set using iterative reconstruction software and will be reoriented into transaxial, coronal and sagittal slices. Attenuation corrected scans will be read. The images will be reviewed in all standard planes (3-dimensional computer display) and a maximum intensity projection (MIP) image. At least 2 parameters will be investigated as measures of tumor response to therapy: SUVmax and SUVmean. Changes in these parameters between the baseline and follow up scans will be used as a measure of effect of study drug. The data analysis equipment and software for nuclear medicine imaging is continuously changing and evolving. As new imaging analysis methods are developed, we will apply those that are optimal to the imaging purpose.

13.1 CRITERIA FOR REMOVAL FROM STUDY

- Patients may be removed from the study for protocol non-compliance.
- If at any time the patient develops unacceptable toxicity he/she will be removed from study.
- Participants can be removed from the study at any time if the study doctor feels that it is in their best interest to do so.

14.0 BIOSTATISTICS

The primary endpoint of this pilot study is to explore if ribavirin therapy for 2 weeks decreases tumor expression of phosphorylated eIF4E among patients with tonsillar and/or base of tongue squamous cell carcinomas that expresses phosphorylated eIF4E.

Decision Rule: We would consider ribavirin to be interesting for further study in this patient population if we observe at least 4 patients (among 7) in which positive baseline expression of phosphorylated eIF4E becomes negative after ribavirin treatment.

Positive phosphorylated eIF4E overexpression is defined as ≥30% of cells with cytoplasmic and/or nuclear staining by IHC (19, 24). With this 30% cutoff, the probabilities of observing at least 4 patients with negative post-treatment eIF4E expression among the 7 patients included in the study are listed below:

True probability that ribavirin inhibits eIF4E expression	**Probability of observing the inhibition in at least 4 of the 7 patients**
.40	.29
.50	.52
.60	.71
.70	.87
.80	.97

For each of the patients tested, we will tabulate pretreatment and posttreatment tumor expression of phosphorylated eIF4E, of molecules that are regulated by eIF4E (p16, p21, EGFR), HPV-16 oncoproteins (E6 and E7) and FDG-PET/CT scans, as well as percentage change between pretreatment and posttreatment levels.

Toxicity data (AEs, laboratory data and vital sign data) will be collected and listed individually per patient according to CTCAE version 4.0.

Subjects who take < 10 days of ribavirin prior to surgery (or research biopsy) will be considered inevaluable and will be replaced. The expected accrual rate is 1 to 2 patients per month, so we expect the study to be completed in 4 to 7 months.

Note: If the post-treatment tumor specimen cannot be obtained (eg, technical issues or patient withdraws consent) or if insufficient/inadequate material is available for immunostaining of phosphorylated eIF4E, the subject will be deemed inevaluable and will be replaced. If there is insufficient/inadequate tissue to perform the other immunostains discussed in the secondary aims, this would not be considered a protocol violation and the subject would not need to be replaced as long as the patient was evaluable for the primary endpoint of this pilot study.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb5.mskcc.org/intranet/_assets/_tables/content/359709/DSMPlans07.pdf*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol

monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol is assessed for it's level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.1 PROTECTION OF HUMAN SUBJECTS

Inclusion of Children in Research

This protocol/project does not include children because the number of children is limited and because the majority are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

17.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.orgcontaining the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE

- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

17.3 Risks, Benefits, Toxicities/side effects

Potential risks to human subjects include drug related toxicity, pain and discomfort associated with ribavrin side effects (Section 5 and Section 11), phlebotomy, and possible psychological discomfort from the stresses associated with obtaining imaging studies. There also is a small potential risk associated with low-level radiation exposure from the research FDG PET-CT scan in this study. All efforts will be made to avoid any complication by completely reviewing patients' symptoms, providing appropriate management, and monitoring blood tests.

For patients who undergo research biopsy, this may be associated with discomfort at the biopsy site and minor bleeding or infection at the biopsy site.

If an adverse medical event occurs, the patient will first contact the primary oncologist or the Principal Investigator. At nights and on weekends, there is an oncology physician on call at all times. Patients may either call or come directly to the urgent care center at Memorial Hospital (or to their local emergency room) to be seen. Patients suffering serious adverse reactions must be carefully followed and all follow-up information also recorded.

17.4 Alternatives/options

Participation in this trial is voluntary. Patients may opt not to participate in this study prior to the planned surgery.

17.5 Financial Costs/Burdens

The patient will be responsible for all costs related to treatment and complications of treatment. Costs to the patient (third party insurer) will include the costs routine blood tests and diagnostic studies, office visits, baseline EKG, doctor's fees, and any hospitalizations. Patients will not be charged for any research tests performed on research specimens. Ribavirin is purchased with Head and Neck DMT research funds and with not billable to research participants. Post-treatment FDG PET-CT scan, if obtained, is paid for Head and Neck DMT research funds and is not billable to research participants.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the

Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Appendix A - Pill Diary