UCSF Helen Diller Family Comprehensive Cancer Center

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Pharmacodynamic effects and predictive biomarkers of JAK/STAT inhibition with ruxolitinib in operable head and neck cancer: a window trial

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Principal Investigator (Sponsor-Investigator)
William Ryan, MD
University of California San Francisco
[redacted]
Telephone: [redacted]
E-mail: [redacted]

Co-Principal Investigator
Patrick Ha, MD
University of California San Francisco
[redacted]
Telephone: [redacted]
E-mail: [redacted]

Statistician
Jimmy Hwang, PhD

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Protocol Signature Page

Protocol No.: 16201

1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Institutional Review Board (IRB), and Data Safety Monitoring Committee (DSMC).

2. I will conduct the study in accordance with applicable IRB requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.

3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.

4. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.

5. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

UCSF Principal Investigator / Study Chair

William Ryan, MD

_____
Printed Name

_____ _____
Signature Date

Protocol Signature Page – Participating Sites

Protocol No.: 16201

Participating Site(s)

Principal Investigator Name: Julie Bauman, MD, MPH
Institution Name: University of Arizona Cancer Center
Address: [redacted]
Telephone: [redacted]
E-mail: [redacted]

I have read this protocol and agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), Institutional Review Board regulations, and all national, state and local laws and/or requirements of the pertinent regulatory requirements.

Principal Investigator	**Site**
Julie Bauman, MD, MPH	**University of Arizona**
Printed Name	Institution Name
Signature	Date

Abstract

Title	Pharmacodynamic effects and predictive biomarkers of JAK/STAT inhibition with ruxolitinib in operable head and neck cancer: a window trial
Patient population	Patients with de novo or recurrent stage I-IVa head and neck squamous cell carcinoma (HNSCC) who are planned for definitive surgery.
Rationale for Study	Head and neck squamous cell carcinoma (HNSCC) is responsible for over 11,500 deaths in the United States alone each year, and despite advances in surgical, radiation, and chemotherapy treatment, overall survival rates have only increased incrementally over the past few decades. Aberrant STAT regulation is commonly seen in HNSCC, although direct pharmacologic targeting of this family of transcriptional factors has proven difficult. However, the Janus kinases (JAK) have been shown to modulate the activity of signal transducer and activator of transcription (STAT)-3 in HNSCC preclinical models, and small molecule JAK inhibitors have recently been developed. The potential utility of JAK modulation in HNSCC has not yet been tested.
Primary Objective	• To identify baseline and/or pharmacodynamic biomarkers of response to ruxolitinib, based upon association with quantitative change in tumor size (ΔTumor size) following 14-21 days of neoadjuvant ruxolitinib in patients with operable HNSCC as determined by quantitative ΔTumor size

Secondary Objectives	• To describe the tolerability of brief neoadjuvant exposure to ruxolitinib
	• To assess the effect of ruxolitinib on the tumoral Ki-67 proliferation index
	• To evaluate additional candidate biomarkers of ruxolitinib response or resistance in HNSCC patients as determined by quantitative ΔTumor size, including:
	  ○ Baseline activation and/or modulation of additional JAK/STAT3 signaling pathway proteins
	  ○ Baseline SHP-2 overexpression
	  ○ Reverse-phase protein array (RPPA) will be conducted on paired pre- and post-treatment tissue as a source of unbiased biomarker discovery

Study Design	This is a multicenter cohort study designed to assess the effect of the JAK 1/2 inhibitor ruxolitinib on tumor proliferation, size, and biomarker expression.
Number of patients	Projected accrual is 45 patients for this study, for a total of 23 biomarker-evaluable patients.
Duration of Therapy	Patients may continue treatment for up to 4 weeks from the time of study entry to time of planned surgery.
Duration of Follow up	12-weeks post-operation, as deemed clinically necessary.
Duration of study	The study will reach completion 24 months from the time the study opens to accrual.
Study Drugs	Ruxolitinib, JAKAFI®, INCB018424 (JAK 1/2 inhibitor) 20mg (or 15mg) PO BID.
Safety Assessments	The safety of this window intervention will be reported descriptively, including tabulation of toxicities according to NCI CTCAE v.4, surgical complications, and length of hospital stay.
Efficacy Assessments	Efficacy of neoadjuvant ruxolitinib in patients with operable HNSCC as determined by quantitative ΔTumor size reflective of a 35% reduction.

List of Abbreviations

AE	adverse event
AUC	area under the curve
BUN	blood urea nitrogen
CBC	complete blood cell (count)
CR	complete response
CRC	Clinical Research Coordinator
CRF	case report form
CSF	cerebral spinal fluid
CT	computerized tomography
CTCEA	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trial Management System
DFS	disease-free survival
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HGB	hemoglobin
ICH	International Conference on Harmonization
IND	investigational new drug application
IRB	Institutional Review Board
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
ORR	overall response rate
PD	disease progression
PK	pharmacokinetics
PO	*Per os* (by mouth, orally)
PR	partial response
PRC	Protocol Review Committee (UCSF)
QOL	Quality of Life
RBC	red blood cell (count)
SD	stable disease
WBC	white blood cell (count)

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1 Introduction

1.1 Background on Indication

Head and Neck Cancer

HNSCC is the sixth leading cancer worldwide, with more than 600,000 cases anticipated in 2012.[1,2] In the United States, the 2012 disease burden included 52,600 new cases and 11,500 deaths.[2] Despite advances in surgical and radiotherapy techniques, as well as integration of chemotherapy into multimodality treatment paradigms, HNSCC is frequently lethal. Five-year overall survival (OS) is 40-60% and has increased only incrementally since 1990.[3] Improved prognosis is largely attributable to the emerging epidemic of oral human papillomavirus (HPV) infection. An increasing proportion of oropharyngeal HNC is driven by oncogenic HPV, rather than the classic risk factors of tobacco and alcohol; HPV etiology is associated with improved survival after conventional treatments.[4,5] Although two distinct causes of HNSCC exist, environmental carcinogenesis or transformation by HPV oncogenes, both etiologies are associated with aberrant STAT 3 signaling.[6-8] The association of STAT3 hyperactivation with poor prognosis[9] and resistance to standard therapies makes it a compelling target in HNSCC.

1.2 Background on Ruxolitinib

JAKAFI®, INCB018424
Despite the importance of STAT function in a number of pathologic processes, direct pharmacologic inhibition of STAT is difficult. However, inhibition of upstream regulators of STAT function via the JAK proteins has met with more success. A number of JAK inhibitors have progressed to various stages of drug development, including a handful that have gained FDA approval. One molecule, ruxolitinib, is an inhibitor of JAK1 and JAK2, and has been approved by the FDA for treatment of myelofibrosis.

Preclinical Anti-tumor Activity
Ruxolitinib represents a novel, potent, and selective inhibitor of *JAK1* and *JAK2.* Ruxolitinib potently inhibits *JAK1* and *JAK2* [half maximal inhibitory concentration (IC50) 0.4 to 1.7 nM], yet it does not significantly inhibit (< 30% inhibition) a broad panel of 26 kinases when tested at 200 nM (approximately 100 times the average IC50 value for JAK enzyme inhibition) and does not inhibit *JAK3* at clinically relevant concentrations. Pharmacological data obtained in *in vivo* model systems support the potential utility of orally administered INCB018424 in the treatment of malignancies, including myeloproliferative disorders such as PMF, PPV-MF and PET-MF. Ruxolitinib retains activity against the *JAK2V617F* mutant and is effective in reducing spleen size in mice inoculated with cells carrying this mutation.

Preclinical Toxicology
The toxicologic and toxicokinetic profiles of ruxolitinib were characterized in single and repeat oral dose studies of up to 6 months in duration in rats and dogs. Genetic toxicology, safety pharmacology, and embryo-fetal toxicology studies have also been conducted.

In a 6-month study in rats, doses up to 60 mg/kg/day were evaluated. An adverse decrease in body weight was noted in male, but not in female rats. A dose related decrease in lymphocytes was noted. Minimal-to-mild lymphoid depletion in spleens and in mandibular lymph nodes was noted at the 60 mg/kg/day dose level; at lower doses, lymphoid tissues were within normal histologic limits. The no-observed-adverse-effect level (NOAEL) for oral administration of ruxolitinib for 26 weeks was 30 mg/kg/day for the males (unbound AUC 0.119 μM*h), related to

adverse effects on body weight at 60 mg/kg/day, and 60 mg/kg/day for female rats (unbound AUC 4.64 μM*h). (Lower parent drug levels in male rats are related to male rat specific isozymes 2C11, 2C13 and CYP3A2 that metabolize ruxolitinib to active metabolites).

In the 6 month dog study, doses studied included 0.5, 2.5, 5, and 10 mg/kg/day. Demodectic mange, lymphopenia, eosinopenia, decreases in erythron parameters, moderate to severe cellular depletion of lymphoid tissues, bacterial pneumonia, viral-induced papillomas, microscopic invasive demodectic mange, and prostate hypoplasia/atrophy were seen in dogs that received high doses. Deaths attributed to bacterial pneumonia occurred in 3 of 14 dogs given 10 mg/kg/day. Demodectic mange was observed in several animals given 5 mg/kg/day and, microscopically, in one animal given 2.5 mg/kg/day. These events most likely reflect a response to the immunosuppressive effects of ruxolitinib. All tissues were normal in the 0.5 mg/kg/day group. The NOAEL dose was defined at 2.5 mg/kg/day (unbound AUC 0.76 μM*h) due to minimal findings, which were not present in recovery animals. The low dose, 0.5 mg/kg/day, was defined as the no-observed effect-level (NOEL). Additional details regarding dog toxicology studies are available in the Investigator Brochure (IB).

Ruxolitinib was not genotoxic in the bacterial mutagenicity assay, the *in vitro* chromosome aberration assay, or the *in vivo* micronucleus assay in rats. In embryo-fetal assessments in rat and rabbit, maternal toxicity and minimal embryo-fetal toxicity were noted at the highest doses evaluated. Ruxolitinib was not teratogenic in either rat or rabbit. The NOAEL dose for the rat and rabbit study was 30 mg/kg/day. Additional toxicology and safety pharmacology information is available in the IB.

Clinical Pharmacokinetics (PK)
Following oral, single-dose administration of ruxolitinib capsules in the fasting state, ruxolitinib was absorbed rapidly, typically attaining peak plasma concentrations within 1 to 3 hours after administration for all doses. After attaining C_{max}, the ruxolitinib plasma concentrations declined with a mean terminal-phase disposition ($t_{1/2}$) of approximately 3-5 hours. The mean ruxolitinib C_{max} and AUC increased with approximately linear proportionality to dose for the entire dose range evaluated of 5 to 200 mg. There was no significant food effect on absorption or exposure. Therefore, ruxolitinib can be dosed without regard to meals.

No accumulation was seen following administration of repeated oral doses of 15 to 50 mg ruxolitinib twice daily (BID), and 50 to 100 mg ruxolitinib once daily for 10 days. PK parameters were similar to those seen following the administration of single doses. Ruxolitinib is metabolized in the liver by the cytochrome (CYP) P450 metabolizing enzyme system, predominantly by the 3A4 isozyme. The effects of the potent CYP3A4 inhibitor ketoconazole on the pharmacokinetics (PK) and pharmacodynamics (PD) of ruxolitinib administered as single oral doses shows that with concomitant dosing of ketoconazole, the observed AUC increase is approximately 2-fold, and the calculated Equivalent Constant Concentration (ECC) for the pSTAT3 PD effect was also increased approximately 2-fold. Thus, a dose reduction of ~ 50% for ruxolitinib is appropriate for participants who take ketoconazole or other potent CYP3A4 inhibitors as concomitant medication (see Section 9.8.1). A more modest effect on the PK parameters of ruxolitinib was demonstrated with concomitant dosing of the moderate CYP3A4 inhibitor erythromycin. No dose adjustments were necessary when ruxolitinib was co-administered with erythromycin, or by extension, with other moderate or weak inhibitors of CYP3A4, including grapefruit juice.

An open-label study to assess the effect of CYP3A4 inducers on ruxolitinib pharmacokinetics and pharmacodynamics revealed that, as expected, rifampin significantly decreased the exposure to ruxolitinib. However, essentially no difference in cytokine-induced STAT3 phosphorylation was observed with or without rifampin induction. This suggests that CYP3A4 induction with rifampin results in metabolism of ruxolitinib to active metabolites that also inhibit JAKs. Increased levels of active metabolites were seen with rifampin dosing. These data indicate that the dose of ruxolitinib need not be modified when dosed with CYP3A4 inducers. However, during the study, use of potent CYP3A4 inducers (rifampin and St. John's Wort) is prohibited. Use of moderate CYP3A4 inducers (rifabutin, carbamazepine, phenytoin) is discouraged, and investigators should seek alternatives where possible. No dose adjustment will be used when these moderate CYP3A4 inducers are co-administered with study drug or open label ruxolitinib. Any concomitant use of CYP3A4 inducers must be documented.

Ruxolitinib was given as a single 25 mg dose to participants with varying degrees of renal function, including normal (calculated creatinine clearance (CrCl) >80 mL/min), mild (CrCl 50-80 mL/min), moderate (CrCl 30-49 mL/min) and severe (CrCl <30 mL/min) renal impairment as well as participants on dialysis and the PK and PD parameters measured. There was no statistically significant effect of mild to severe impairment of renal function on the PK or PD parameter; participants requiring dialysis showed decreased ruxolitinib clearance. Participants with serum creatinine exceeding 1.5 times the institutional upper limit of normal (ULN) will be excluded from the study.

Randomized Studies
Two randomized Phase 3 studies (Studies 1 and 2) were conducted in patients with myelofibrosis (either primary myelofibrosis, post-polycythemia vera myelofibrosis or post-essential thrombocythemia-myelofibrosis). In both studies, patients had palpable splenomegaly at least 5 cm below the costal margin and risk category of intermediate 2 (2 prognostic factors) or high risk (3 or more prognostic factors) based on the International Working Group Consensus Criteria (IWG).

The starting dose of ruxolitinib was based on platelet count. Patients with a platelet count between 100 and 200 X 10^9/L were started on ruxolitinib 15 mg twice daily and patients with a platelet count greater than 200 X 10^9/L were started on ruxolitinib 20 mg twice daily. Doses were then individualized based upon tolerability and efficacy with maximum doses of 20 mg twice daily for patients with platelet counts between 100 to less than or equal to 125 X 10^9/L, of 10 mg twice daily for patients with platelet counts between 75 to less than or equal to 100 X 10^9/L, and of 5 mg twice daily for patients with platelet counts between 50 to less than or equal to 75 X 10^9/L.

<u>Study 1</u>
Study 1 was a double blind, randomized, placebo-controlled study in 309 patients who were refractory to or were not candidates for available therapy. The median age was 68 years (range 40 to 91 years) with 61% of patients older than 65 years and 54% were male. Fifty percent (50%) of patients had primary myelofibrosis, 31% had post-polycythemia vera myelofibrosis and 18% had post-essential thrombocythemia myelofibrosis. Twenty-one percent (21%) of patients had red blood cell transfusions within 8 weeks of enrollment in the study. The median hemoglobin count was 10.5 g/dL and the median platelet count was 251 X 109/L. Patients had a median palpable spleen length of 16 cm below the costal margin, with 81% having a spleen length 10 cm or greater below the costal margin. Patients had a median spleen volume as

measured by magnetic resonance imaging (MRI) or computed tomography (CT) of 2595 cm^3 (range 478 cm^3 to 8881 cm^3). (The upper limit of normal is approximately 300 cm^3).

Patients were dosed with ruxolitinib or matching placebo. The primary efficacy endpoint was the proportion of patients achieving greater than or equal to a 35% reduction from baseline in spleen volume at Week 24 as measured by MRI or CT.

Secondary endpoints included duration of a 35% or greater reduction in spleen volume and proportion of patients with a 50% or greater reduction in Total Symptom Score from baseline to Week 24 as measured by the modified Myelofibrosis Symptom Assessment Form (MFSAF) v2.0 diary.

Study 2
Study 2 was an open-label, randomized study in 219 patients. Patients were randomized 2:1 to ruxolitinib versus best available therapy. Best available therapy was selected by the investigator on a patient-by-patient basis. In the best available therapy arm, the medications received by more than 10% of patients were hydroxyurea (47%) and glucocorticoids (16%). The median age was 66 years (range 35 to 85 years) with 52% of patients older than 65 years and 57% were male. Fifty-three percent (53%) of patients had primary myelofibrosis, 31% had post-polycythemia vera myelofibrosis and 16% had post-essential thrombocythemia myelofibrosis. Twenty-one percent (21%) of patients had red blood cell transfusions within 8 weeks of enrollment in the study. The median hemoglobin count was 10.4 g/dL and the median platelet count was 236 X 109/L. Patients had a median palpable spleen length of 15 cm below the costal margin, with 70% having a spleen length 10 cm or greater below the costal margin. Patients had a median spleen volume as measured by MRI or CT of 2381 cm^3 (range 451 cm^3 to 7765 cm^3).

The primary efficacy endpoint was the proportion of patients achieving 35% or greater reduction from baseline in spleen volume at Week 48 as measured by MRI or CT.

A secondary endpoint in Study 2 was the proportion of patients achieving a 35% or greater reduction of spleen volume as measured by MRI or CT from baseline to Week 24.

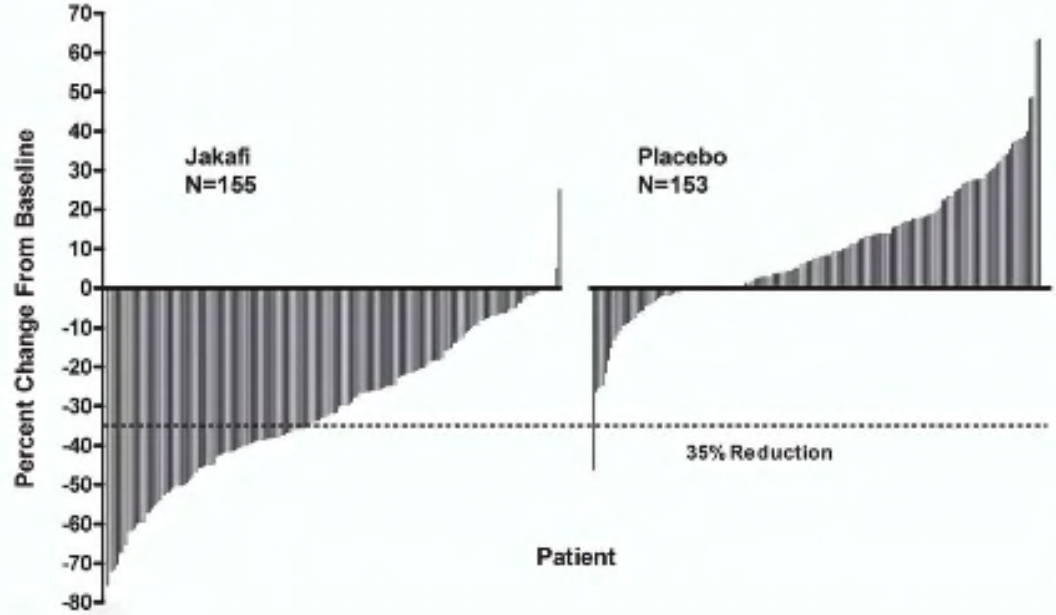
Study 1 and 2 Efficacy Results
Efficacy analyses of the primary endpoint in Studies 1 and 2 are presented in Table 1 below. A significantly larger proportion of patients in the ruxolitinib group achieved a 35% or greater reduction in spleen volume from baseline in both studies compared to placebo in Study 1 and best available therapy in Study 2. A similar proportion of patients in the ruxolitinib group achieved a 50% or greater reduction in palpable spleen length.

Table 1: Percent of Patients with 35% or Greater Reduction from Baseline in Spleen Volume at Week 24 in Study 1 and at Week 48 in Study 2 (Intent to Treat)

	Study 1		**Study 2**	
	Ruxolitinib (N=155)	Placebo (N=154)	Ruxolitinib (N=146)	Placebo (N=73)
Time Points	**Week 24**		**Week 48**	
Number (%) of Patients with Spleen Volume Reduction by 35% or More	65 (41.9)	1 (0.7)	41 (28.5)	0
P-value	<0.0001		<0.0001	

Figure 1 shows the percent change from baseline in spleen volume for each patient at Week 24 (ruxolitinib N=139, placebo N=106) or the last evaluation prior to Week 24 for patients who did not complete 24 weeks of randomized treatment (ruxolitinib N=16, placebo N=47). One (1) patient (placebo) with a missing baseline spleen volume is not included.

Figure 1: Percent Change from Baseline in Spleen Volume at Week 24 or Last Observation for Each Patient (Study 1)



In Study 1, myelofibrosis symptoms were a secondary endpoint and were measured using the modified Myelofibrosis Symptom Assessment Form (MFSAF) v2.0 diary. The modified MFSAF is a daily diary capturing the core symptoms of myelofibrosis (abdominal discomfort, pain under left ribs, night sweats, itching, bone/muscle pain and early satiety). Symptom scores ranged from 0 to 10 with 0 representing symptoms “absent” and 10 representing “worst imaginable” symptoms. These scores were added to create the daily total score, which has a maximum of 60.

Table 2 presents assessments of Total Symptom Score from baseline to Week 24 in Study 1 including the proportion of patients with at least a 50% reduction (i.e., improvement in symptoms). At baseline, the mean Total Symptom Score was 18.0 in the ruxolitinib group and 16.5 in the placebo group. A higher proportion of patients in the ruxolitinib group had a 50% or greater reduction in Total Symptom Score than in the placebo group, with a median time to response of less than 4 weeks.

Table 2: Improvement in Total Symptom Score

	Ruxolitinib (N=148)	**Placebo (N=152)**
Number (%) of Patients with 50% or Greater Reduction in Total Symptom Score by Week 24	68 (45.9)	8 (5.3)
P-value	< 0.0001	

Figure 2 shows the percent change from baseline in Total Symptom Score for each patient at Week 24 (ruxolitinib N=129, placebo N=103) or the last evaluation on randomized therapy prior to Week 24 for patients who did not complete 24 weeks of randomized treatment (ruxolitinib N=16, placebo N=42). Results are excluded for 5 patients with a baseline Total Symptom Score of zero, 8 patients with missing baseline and 6 patients with insufficient post-baseline data.

Figure 2: Percent Change from Baseline in Total Symptom Score at Week 24 or Last Observation for Each Patient (Study 1)

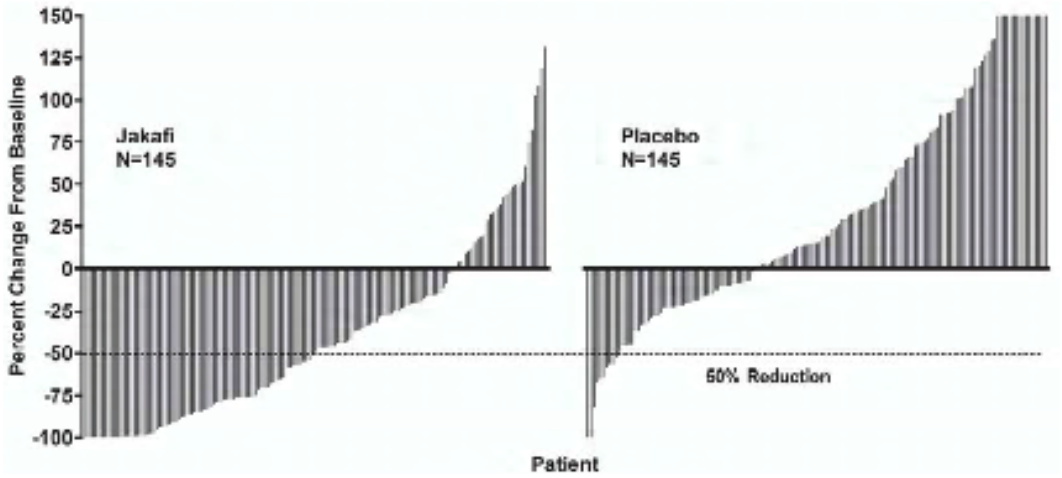
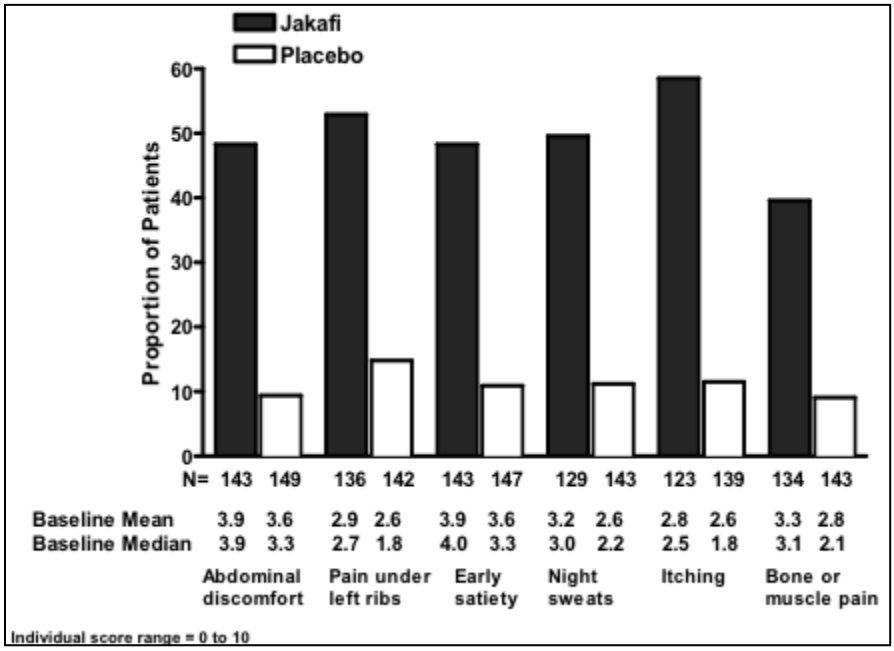


Figure 3: Proportion of Patients With 50% or Greater Reduction in Individual Symptom Scores at Week 24

Figure 3. The proportion of patients with at least a 50% improvement in each of the individual symptoms that comprise the Total Symptom Score indicating that all 6 of the symptoms contributed to the higher Total Symptom Score response rate in the group treated with ruxolitinib.



Safety of Ruxolitinib in Clinical Studies

The safety of ruxolitinib was assessed in 617 patients in six clinical studies with a median duration of follow-up of 10.9 months, including 301 patients with myelofibrosis in two Phase 3 studies.

In these two Phase 3 studies, patients had a median duration of exposure to ruxolitinib of 9.5 months (range 0.5 to 17 months), with 88.7% of patients treated for more than 6 months and 24.6% treated for more than 12 months. One hundred and eleven (111) patients started treatment at 15 mg twice daily and 190 patients started at 20 mg twice daily.

In a double blind, randomized, placebo-controlled study of ruxolitinib, 155 patients were treated with ruxolitinib. The most frequent adverse drug reactions were thrombocytopenia and anemia. Thrombocytopenia, anemia and neutropenia are dose related effects. The three most frequent non-hematologic adverse reactions were bruising, dizziness and headache.

Discontinuation for adverse events, regardless of causality, was observed in 11.0% of patients treated with ruxolitinib and 10.6% of patients treated with placebo.

Following interruption or discontinuation of ruxolitinib, symptoms of myelofibrosis generally return to pretreatment levels over a period of approximately 1 week. There have been isolated cases of patients discontinuing ruxolitinib during acute intercurrent illnesses after which the patient's clinical course continued to worsen; however, it has not been established whether discontinuation of therapy contributed to the clinical course in these patients. When discontinuing therapy for reasons other than thrombocytopenia, gradual tapering of the dose of ruxolitinib may be considered.

Table 3 presents the most common adverse reactions occurring in patients who received ruxolitinib in the double blind, placebo-controlled study during randomized treatment.

Table 3: Adverse Reactions Occurring in Patients on Ruxolitinib in the Double blind, Placebo-controlled Study during Randomized Treatment

	Jakafi (N=155)			Placebo (N=151)		
Adverse Reactions	**All Grades[a] (%)**	**Grade 3 (%)**	**Grade 4 (%)**	**All Grades (%)**	**Grade 3 (%)**	**Grade 4 (%)**
Bruising[b]	23.2	0.6	0	14.6	0	0
Dizziness[c]	18.1	0.6	0	7.3	0	0
Headache	14.8	0	0	5.3	0	0
Urinary Tract Infections[d]	9.0	0	0	5.3	0.7	0.7
Weight Gain[e]	7.1	0.6	0	1.3	0.7	0
Flatulence	5.2	0	0	0.7	0	0
Herpes Zoster[f]	1.9	0	0	0.7	0	0

[a] National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0
[b] includes contusion, ecchymosis, hematoma, injection site hematoma, periorbital hematoma, vessel puncture site hematoma, increased tendency to bruise, petechiae, and purpura
[c] includes dizziness, postural dizziness, vertigo, balance disorder, Meniere's Disease, labyrinthitis
[d] includes urinary tract infection, cystitis, urosepsis, urinary tract infection bacterial, kidney infection, pyuria, bacteria urine, bacteria urine identified, nitrite urine present
[e] includes weight increased, abnormal weight gain
[f] includes herpes zoster and post-herpetic neuralgia

1.3 Rationale for the Proposed Study

Cumulative preclinical data implicate STAT 3 as a critical target in HNSCC; however, as with other transcription factors, STAT3 has been challenging to selectively and directly target. The recognition that hyperactivated STAT3 may result from increased upstream JAK signaling caused us to investigate JAK 1/2 inhibitors, including AZD1480 and ruxolitinib, in HNSCC preclinical models as described above. Promising preclinical results parallel findings from multiple solid tumors, where AZD1480 abrogated IL-6 dependent STAT3 signaling,[10] justifying translation of JAK 1/2 targeting into human clinical trials. Novel insights into the genomic and epigenetic mechanisms of HNSCC tumor STAT3 activation raise the exciting possibility of biomarker-selection of patients most likely to respond to JAK 1/2 inhibition.

Ruxolitinib is an ATP-competitive, oral, small molecule inhibitor of the JAK1 and JAK2 kinases; it is the first JAK family inhibitor approved by the U.S. Food and Drug Administration (Incyte,

myelofibrosis, 2011).[11] Inhibition of wild type JAK1 and JAK2 respectively has an enzyme IC_{50} of 3.3 (+/- 1.2) and 2.8 (+/- 1.2) nM, concentrations easily achieved by the approved clinical dose.[12] To date, ruxolitinib duplicates the findings of AZD1480 in our HNSCC preclinical models (Figures 4-8), as would be expected from its similar JAK inhibitory and pharmacokinetic profile.

In this study, ruxolitinib will be administered for a short period of 2-3 weeks prior to planned surgical resection of HNSCC. The dose will be the FDA-approved dose in myelofibrosis, as determined by baseline platelet count: 1) Patients with a platelet count ≥ 200,000 will take 20 mg bid (four 5mg tablets in the morning and four 5mg tablets in the evening); 2) participants with a platelet count ≥ 150,000 and < 200,000 will take 15 mg bid (three 5 mg tablets in the morning and three 5 mg tablets in the evening). The brief treatment duration is within the expected window of time that elapses from initial patient evaluation by a surgeon to performance of surgery. In the phase 0 or window trial model, a paired specimen analysis permits *ex vivo* evaluation of target modulation and pharmacodynamic changes in downstream or parallel molecular pathways.[13] This study design is optimal in order to assess the biochemical and immunomodulatory effects of ruxolitinib on HNSCC. Furthermore, predictive biomarkers can be developed as related to a clinical endpoint (ΔTumor size) or a biochemical, pharmacodynamic endpoint (Δ Ki-67).

Ki-67 is a nuclear non-histone protein first characterized in 1991, which is expressed in proliferating human tissue.[14] Down-modulation of Ki-67 is a validated surrogate biomarker in neoadjuvant studies of targeted therapy in breast cancer.[15,16] Changes in Ki-67 observed in a neoadjuvant endocrine therapy study predicted the ultimate long-term disease-free survival advantage in the larger, adjuvant trial, whereas clinical response did not.[16,17] In HNSCC window trials, Ki-67 modulation has not been evaluated directly against clinical outcome.[18-20] An alternate surrogate biomarker to Ki-67, the TUNEL apoptotic index, was evaluated as the primary endpoint in a randomized window trial of lapatinib vs. placebo, prior to standard chemoradiotherapy in HNSCC.[18] Although apoptosis was increased by lapatinib, the increase was not significant against that seen in the placebo group. Notably, the Ki-67 proliferation index was significantly decreased by lapatinib vs. placebo. This is in line with results from our first window study in HNSCC evaluating erlotinib, erlotinib-sulindac or placebo – where Ki-67 reduction was significantly greater in the erlotinib arms vs. placebo.[21] Collectively, these data suggest that proliferation is a worthwhile secondary endpoint for evaluation of biomarker correlations.

The Jak/STAT pathway in Head and Neck Cancer

The STAT family of transcription factors is the central mediator of cellular response to cytokines and growth factors. Aberrant regulation of STAT3 and STAT1 in HNSCC underlies both malignant behavior and immune escape. STAT3 is an oncogenic transcription factor that is hyperactivated (phosphorylated) in a wide variety of malignancies. Studies from UPCI and other laboratories have shown that STAT3 transforms human epithelial cells, thereby meeting the definition of an oncogene. In HNSCC, STAT3 facilitates tumor growth and survival, and mediates resistance to standard therapies including chemoradiation and blockade of the epidermal growth factor receptor (EGFR).[22-26]

While cumulative evidence implicates STAT3 as an important target for cancer treatment, as with other transcription factors, STAT3 has proven challenging to inhibit selectively in humans. Few drugs have been amenable to clinical development. For example, an intra-operative phase 0 trial of a STAT-3 oligonucleotide decoy in HNSCC was completed at UPCI, which reduced

target gene expression; however, the limited half-life and requirement for local injection impede broader clinical development.[19] Increasing insight into the mechanisms of STAT3 hyperactivation in human cancer raises the possibility of new therapeutic avenues, and may identify biomarkers for patient selection.

Although STAT3 is constitutively phosphorylated, activating mutations are not described in HNSCC[27,28] or other human epithelial cancers. Thus, hyperactivated STAT3 likely results from excess activation of positive regulators and/or inactivation of negative regulators. STATs are phosphorylated by upstream growth factor receptors including Src and Janus kinases (JAK), and de-phosphorylated by protein tyrosine phosphatase receptors (PTPR). The activating JAK mutations identified in myeloproliferative disorders are not found in HNSCC.[27,28] In HNSCC, IL-6 stimulates tyrosine phosphorylation of STAT3 through JAK 1 and 2.[29] The recent clinical development of JAK inhibitors, including ruxolitinib, raises the therapeutic plausibility of blocking this autocrine loop. For example, the JAK 1/2 inhibitor, AZD1480, abrogated IL-6 induced STAT3 phosphorylation and suppressed the growth of human solid tumor xenografts with constitutive STAT3 activity.[10] Moreover, a strategy for patient selection is emerging. While activating mutations in JAK or STAT3 are not found in HNSCC, our recent comprehensive mutational profiling of HNSCC detected mutations in the PTPR family of genes in nearly one-third of tumors.[27] Analysis of the 347 HNSCC tumors sequenced to date under the auspices of The Cancer Genome Atlas (TCGA) and our group, confirmed this high incidence of PTPR mutations (104/347, 30%). Further analysis demonstrated that PTPR genes are mutated in a wide array of solid tumors sequenced to date under the auspices of the TCGA (see Table 1). Loss of function mutations in PTPRT and PTPRD lead to increased STAT3 phosphorylation, suggesting a tumor suppressor role.[30,31] Whole exome sequencing (n=374) and reverse-phase protein array data has been analyzed from 212 HNSCC tumors[32]. PTPR mutations were common and were associated with significantly increased phospho-STAT3 expression. Expression of PTPRT mutant proteins induced STAT3 phosphorylation and cell survival, consistent with an oncogenic “driver” phenotype. Computational modeling revealed functional consequences of PTPRT mutations on phosphor-tyrosine-substrate interactions. In addition to genomic aberrations, PTPRT is commonly methylated in HNSCC tumors (60%, 166/279 tumors analyzed to date in the TCGA); expression levels of PTPRT are significantly lower in these methylated tumors. Thus, silencing of key PTPR family genes may underlie STAT3 hyperactivation in HNSCC and other solid tumors. Identification and functional classification of these common genetic and epigenetic PTPR aberrations may permit companion biomarker development to select patients most likely to benefit from JAK inhibitors.

Table 4. Frequency of PTPR Mutations in Human Cancers.

Cancer Type (TCGA)	% Mutated Tumors	
Head & Neck	30.00%	(104/347)
Bladder	41.00%	(41/100)
Breast	10.70%	(54/507)
Cervix	25.00%	(9/36)
Colon	35.30%	(175/496)
Endometrioid	32.30%	(80/248)
Lung Adenocarcinoma	46.50%	(107/230)
Lung Squamous	45.40%	(83/183)
Ovary	14.30%	(65/456)
Prostate	8.40%	(7/83)
Stomach	53.60%	(81/151)

Figure 4: Decrease in pSTAT3$_{Tyr705}$ expression with increase in concentration of Ruxolitinib

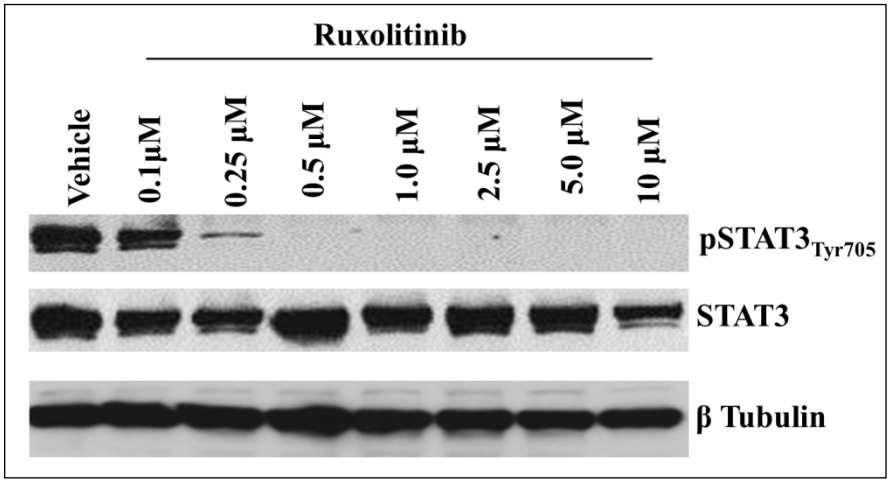
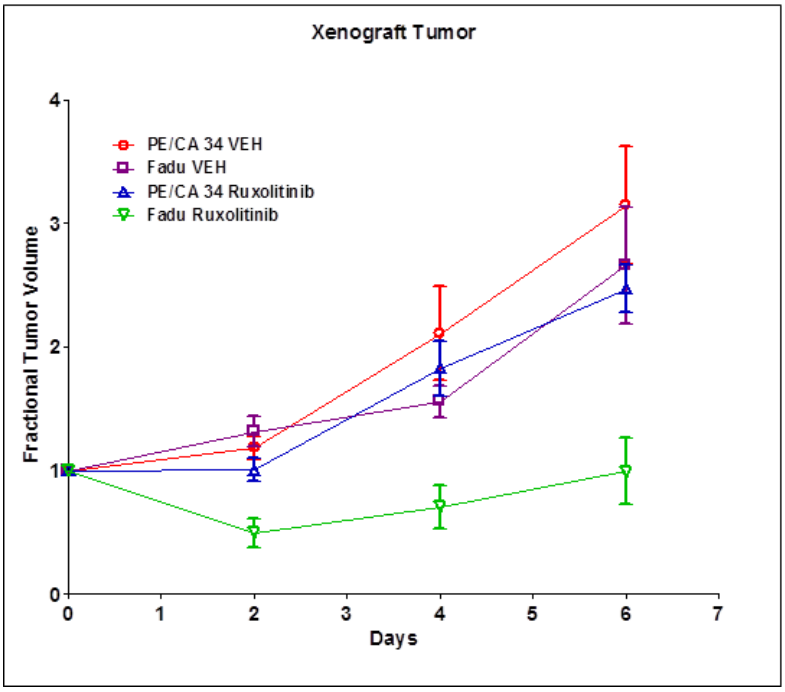


Figure 4. Cal 33 cells were treated with increasing concentrations of Ruxolitinib. After 24h, cells were harvested to obtain cell lysates. Forty micrograms of protein/lane were subjected to electrophoresis and immunoblotted for pSTAT3$_{Tyr705}$ and total STAT3. B-tubulin was used as a loading control.

Recognition that hyperactivated STAT3 likely results from increased signaling through upstream kinases, or loss of de-phosphorylation by silenced PTPRs, initially prompted investigation into the preclinical JAK1/2 inhibitor, AZD1480, in relevant HNSCC preclinical models. AZD1480 inhibited proliferation of eight HNSCC cell lines at low µM concentrations (data not shown). Ruxolitinib is an ATP-competitive, oral, small molecule inhibitor of the JAK1 and JAK2 tyrosine kinases. Inhibition of wild type JAK1 and JAK2 respectively has an enzyme IC_{50} of 3.3 (+/- 1.2) and 2.8 (+/- 1.2) nM.[12] Ruxolitinib blocked phosphorylation of pSTAT3 at Tyrosine 705 in dose-dependent fashion in the Cal33 HNSCC cell line (Figure 4). Furthermore, ruxolitinib inhibited growth of a FaDu HNSCC cell line xenograft, which harbors a loss-of-function *PTPRT* mutation, as compared to FaDu xenografts treated with vehicle control or ruxolitinib-treated PE/CA 34 HNSCC xenografts, which are wild type for *PTPRT* (Figure 4).

Figure 5: PTPRD or PTPRT mutations are found in 30% of HNSCC tumors. PTPRD and PTPRT mutations induce STAT3 activation, and increase sensitivity to JAK 1/2 inhibition.



The PTPRT/D mutations found in HNSCC tumors to date are shown below in Figure 6. Mutations in the PTP catalytic domains (PTPRT and PTPRD) and the fibronectin type 3 domain (PTPRD) have been reported to inhibit cellular functions of the phosphatase activity of these proteins.[31,33] PTPRO was recently shown to behave as a tumor suppressor and decrease pSTAT3 in liver cancer.[34] It is noteworthy that activating mutations of upstream mediators of STAT3 in HNSCC including EGFR, JAK, and Src were not detected in our HNSCC cohort. Furthermore, there was no evidence for association of PTPR mutations with HPV status. Published data[32] illustrate that HNSCC tumors bearing PTPR mutations demonstrate higher pSTAT3 expression compared to PTPR wild-type. HNSCC cells transfected with a PTPRT mutation (A1022E or R1040L) demonstrate increased growth relative to EGFP-vector control or wild-type PTPR.

Figure 6. Schematics showing mutations of PTPRT and PTPRD found in HNSCC patient tumors.

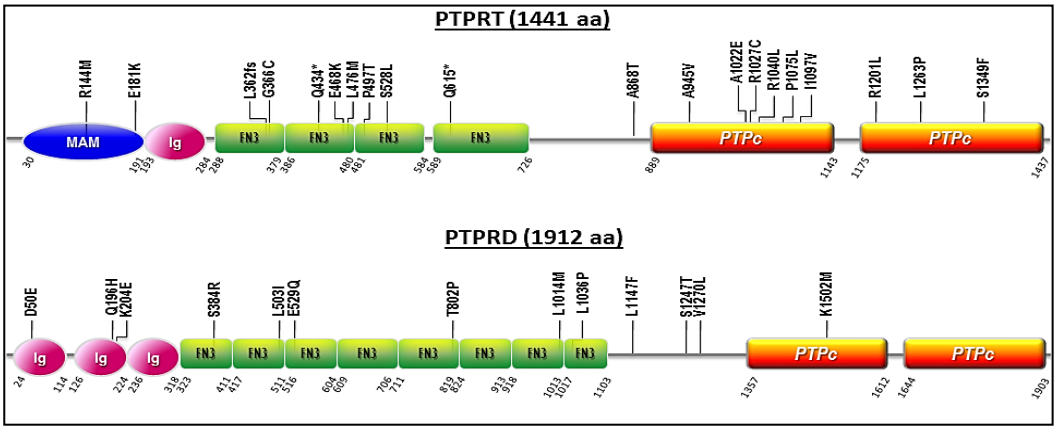
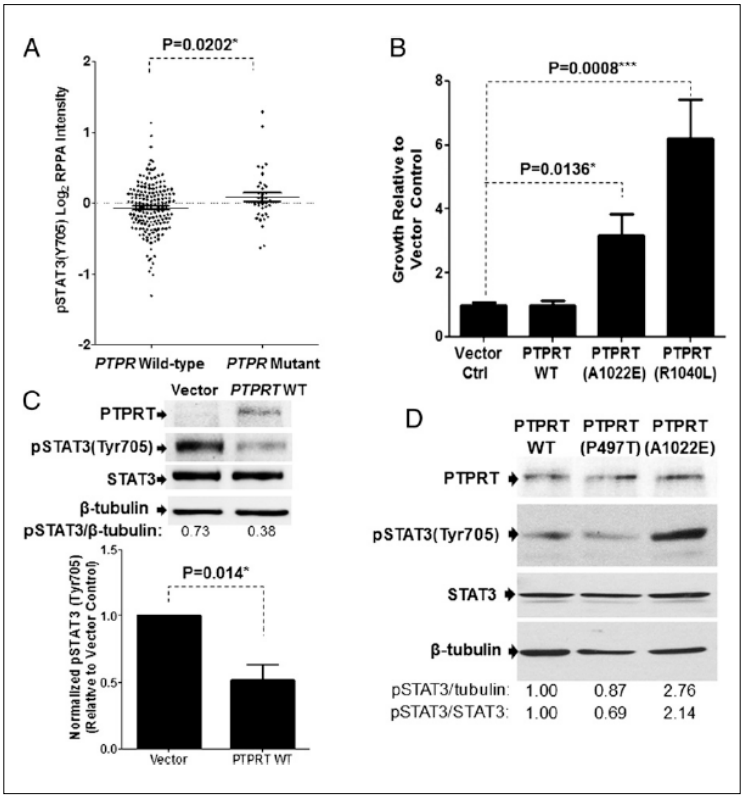


Figure 6. Keys: ***Blue***: Meprin/A5/PTPmu domain (MAM); ***Green***: Fibronectin type 3 domain (FN3); ***Orange***: Protein tyrosine phosphatase catalytic domain (PTPc); ***Pink***: immunoglobulin (Ig) or Ig-like domain.

Figure 7. PTPR mutations correlate with STAT3 tyrosine phosphorylation in HNSCC tumors and enhance survival and STAT3 phosphorylation in HNSCC cells.

Figure 7. (A) Significant increase in p-TAT3(Y705) expression (intensity plot in log2 scale; protein array data from The Cancer Proteome Atlas) in HNSCC patient tumors harboring PTPRD/J/K/M/O/S/T mutation (n =37) vs. tumors without PTPRT mutation (n = 171) (P = 0.0202*). (B) Serum-dependent PCI-52-SD1 cells stably expressing EGFP-vector control, wild-type PTPRT, or PTPRT(A1022E) or (R1040L) mutants were assessed by MTT assay for growth in the absence of serum. Cumulative growth relative to vector control (n ≥ 9) is shown. (C) Stable expression of wild-type PTPRT reduced basal p-STAT3(Tyr705) expression in PCI-52-SD1 cells. Graph showing cumulative results of p-STAT(Y705) levels in these cells from five independent experiments. (D) CAL-33 cells were transiently transfected with a representative PTPase domain mutation (A1022E) and an FN3-domain mutation (P497T). Expression levels of p-STAT3 (Tyr705) were detected by Western blotting. The PTPase domain mutation, but not the FN3-domain mutation, increased p-STAT3(Tyr705) expression. Similar results were observed in three independent experiments.



Preliminary data show that HNSCC cells transfected with a PTPRD mutation (P311T) demonstrated increased baseline STAT3 activation compared to vector controls (data not shown), and decreased survival and STAT3 promoter activity when treated with AZD1480 compared with controls (Figure 8).

Figure 8. Expression of PTPRD mutant in HNSCC cells

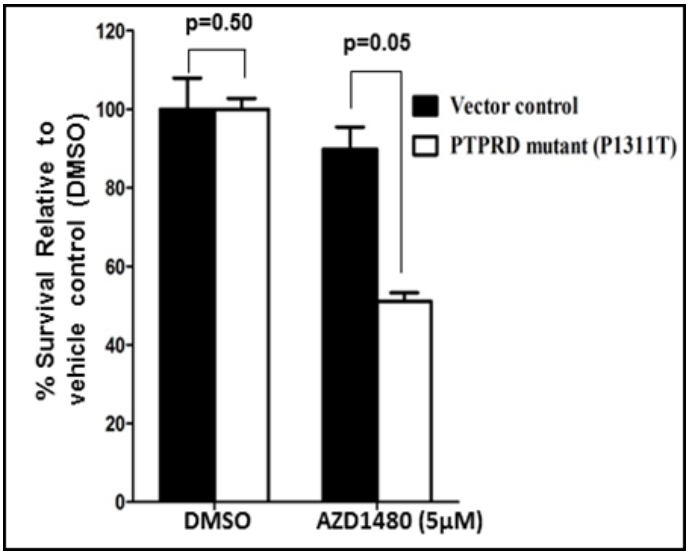


Figure 8. UM-SCC-1 cells expressing mutant PTPRD (P1311T) or vector control were treated with AZD1480 (5 μM) or DMSO (vehicle) followed by MTT assay to determine % survival relative to vehicle control. Results are mean +/- SEM, performed in triplicate.

Finally, *PTPRT*, the most commonly mutated *PTPR* in HNSCC is methylated in 60% of samples analyzed to date by the TCGA where tumors with methlyated *PTPRT* express significantly lower levels of this protein. Further investigation in our lab showed that demethylation of a cell line with methylated *PTPRT* decreased pSTAT3 expression (data not shown)

1.4 Correlative Biomarker Analysis

1.4.1 Primary Analysis

Correlative biomarker analysis will be performed on paired pre-treatment specimens (tumor biopsies and blood) and post-treatment samples to determine the modulation of biomarkers by ruxolitinib. Most importantly, biomarkers within the JAK/STAT signaling pathway, including JAK and STAT phosphorylation, PTPR mutation and methylation, inflammatory cytokines and HLA/APM component expression will be assessed to determine their correlation with clinical response to ruxolitinib. Tissue microarrays will be generated from formalin-fixed, paraffin-embedded (FFPE) specimens, and these will be used for immunohistochemistry to assess tissue histology, Ki67 labeling, and expression of JAK/STAT pathway proteins, including phosphorylated and non-phosphorylated forms of JAK1, JAK2, STAT1, STAT3, cyclin D1 and Src. PTPR mutations and methylation will be determined by Sanger sequencing/Sequenom[31] and methylation-specific PCR, respectively. Finally, HLA/APM component expression will be evaluated using IHC or intracellular flow cytometry for TAP1, TAP2, LMP2 and calreticulin, as described[35]. Remaining specimens after these studies have been completed will be used for additional analysis.

1.4.2 Additional Analysis

To determine if expression of serum proteins or DNA polymorphisms predict response to ruxolitinib targeting, paired serum (baseline, post-treatment) and baseline peripheral blood mononuclear cells (PBMCs) will be isolated. Blood will be processed for serum and isolation of PBMCs. Serum will be analyzed for proteomic markers using mass spectroscopy and luminex ELISA assays. DNA will be isolated from PBMC samples for SNP analysis. Tissue will be processed for DNA, fluorescence in situ hybridization (FISH), tissue microarrays, and

immunohistochemistry microarrays. Both uniplex and multiplex serum cytokine profile analysis, for correlation with other biomarkers and clinical responsiveness will be performed. For specific cytokines of interest, including IL-6, ELISA kits will be used. For multiplex analysis, the technology developed by Luminex Corporation (Austin, TX, http://www.luminexcorp.com) will be used. It enables a laboratory to simultaneously measure up to 100 analytes in a single microplate well, using very small sample volumes (less than 50 microliters). Previous studies have shown that a multi-marker panel offering the highest diagnostic power was comprised of 22 biomarkers, including EGF, EGFR, Cyfra 21-1, AFP, IL-8, MMP-3, CA 72-4, CA 15-3, MMP-2, TNF-RI, IL-7, HCGb, MIF, Mesothelin, MCP-1, IL-1Ra, FGFb, CEA, sVCAM-1 , tPAI 1, RANTES, IGFBP-1. Statistical analysis using our novel ADE algorithm resulted in a sensitivity of 84.5%, specificity of 98%, and 92% of patients with active head and neck cancer correctly classified from a cross-validation serum set[36]. Further proteomic analysis will be performed using IHC microarrays.

2 Objectives of the Study

2.1 Primary Objective

To identify baseline and/or pharmacodynamic biomarkers of response to ruxolitinib based upon association with quantitative change in tumor size (ΔTumor size) following 14-21 days of neoadjuvant ruxolitinib in patients with operable HNSCC. We hypothesize that ΔTumor size will correlate with five prioritized, predictive biomarkers which will be designated for specialized alpha spending: 1) baseline expression of pSTAT3; 2) change in expression of pSTAT3; 3) change in expression of the STAT3 target gene cyclin D1; 4) baseline silencing of protein tyrosine phosphatase receptors D or T (PTPRD or PTPRT) by mutation or methylation; 5) baseline elevation in peripheral inflammatory markers (C-reactive protein [CRP]).

2.2 Secondary Objectives

- To assess preliminary efficacy of neoadjuvant ruxolitinib in patients with operable HNSCC as determined by quantitative ΔTumor size
- To describe the tolerability of brief neoadjuvant exposure to ruxolitinib
- To assess the effect of ruxolitinib on the tumoral Ki-67 proliferation index
- To evaluate additional candidate biomarkers of ruxolitinib response or resistance in HNSCC patients as determined by quantitative ΔTumor size, including:
    - Baseline activation and/or modulation of additional JAK/STAT3 signaling pathway proteins
    - Baseline SHP-2 overexpression
    - Reverse-phase protein array (RPPA) will be conducted on paired pre- and post-treatment tissue as a source of unbiased biomarker discovery

2.3 Endpoints

2.3.1 Primary Endpoint

- The **primary efficacy endpoint** of the study is clinical ruxolitinib response of quantitative ΔTumor size measured as a proportional percent (range -100% to +100%) from baseline to day 14-21.

2.3.2 Secondary Endpoints

- The size of the tumor will be determined and net ΔTumor measurements used to determine the response.
- The safety of this window intervention will be reported descriptively, including tabulation of toxicities according to NCI CTCAE v.4, surgical complications, and length of hospital stay.
- The Ki-67 proliferative index will be measured in baseline and post-treatment tumor tissue as described, and will serve as a secondary endpoint (pharmacodynamic efficacy).[21] The five prioritized biomarkers will be analyzed for correlations with ΔTumor size, as above. In addition, they will be analyzed for correlations with ΔKi-67.
- Non-prioritized biomarkers will also be evaluated for correlations with ΔTumor size and ΔKi-67. In order to assess biomarker response to ruxolitinib, we will test each component of the biomarker panel for within-patient differences between the pre-treatment and post-treatment biospecimens (tumor tissue and blood) and compute the p-values for each paired test (either a paired t test or the signed rank test). We anticipate a panel of 15 – 20 proteins will be tested for short-term modulation with TMAs. P values from paired data will be adjusted to control the expected false discovery rate by the method of Benjamini and Hochberg. Additional analyses of secondary endpoints will be conducted on an exploratory and hypothesis-generating bases using available material. These endpoints may include qualitative and semi-quantitative scoring of IHC in tissue arrays, gene microarrays and serum protein measures.

3 Study Design

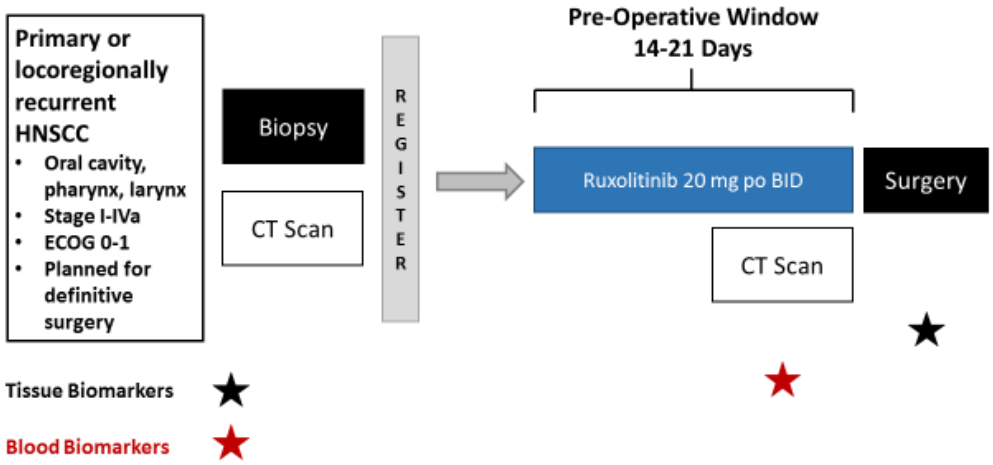
3.1 Characteristics

This is a multicenter cohort study designed to assess the effect of the JAK 1/2 inhibitor ruxolitinib on tumor proliferation, size, and biomarker expression.

Screening: Biopsies of the tumor and peripheral blood sample collection (see section 3.1)

After 14-21 days: Repeat CT scan and peripheral blood collection, followed by planned oncologic surgery with collection of post-treatment tumor specimen

Schema of Proposed Clinical Trial



3.2 Number of Participants

45 patients will be accrued to achieve the target of 23 eligible patients demonstrating treatment compliance, and with quality paired tumor specimens.

3.3 Eligibility Criteria

Surgery will be the primary curative treatment for patients enrolled in this study. Patients should not require any standard induction treatment prior to surgery. Surgery will have to be the best treatment option as determined by the treating physician. Therefore, we will not be delaying chemoradiotherapy or other curative treatment. We plan to include any stage of HNSCC that will be managed by primary surgery, including patients with newly diagnosed disease or locoregional recurrence scheduled for surgical salvage. If surgery is unexpectedly cancelled, the patient will be removed from the study unless there is an accessible lesion for biopsy. Ideally, the pre-treatment biopsy and the intraoperative sample will be obtained from the same site (when there are multiple lesions).

3.3.1 Inclusion Criteria

1. Histologically or cytologically confirmed, primary or recurrent, head and neck squamous cell carcinoma, including variants. Patients must have at least one measureable lesion in accordance with RECIST 1.1 (tumor diameter ≥ 1 cm; short-axis lymph node diameter ≥ 1.5 cm) OR by caliper measurement (tumor diameter ≥ 1 cm). Any diagnostic pretreatment biopsy sample is acceptable including FNA.
2. Primary tumors of any head and neck (oral cavity, oropharynx, hypopharynx, or larynx) site will be included.
3. Surgical resection of head and neck must be planned, either as primary treatment or salvage. Patients must have submitted adequate pre-treatment archival or fresh tissue.
4. Age ≥ 18 years.
5. ECOG performance status 0-2 (See Appendix 1).

6. Women of childbearing potential (WOCBP) must have a negative serum pregnancy test (sensitivity ≤ 25IU HCG/L) within 4 weeks prior to registration and will be repeated within 72 hours prior to the start of study drug administration.
7. Persons of reproductive potential must agree to use and utilize an adequate method of contraception throughout treatment and for at least 12 weeks after study drug is stopped. Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy.
8. Adequate hematologic, renal and hepatic function, as defined by:
    a) Absolute neutrophil count (ANC) ≥ 1,500/ul, platelets ≥ 150,000/ul.
    b) Creatinine ≤ 1.5 x institutional upper limit of normal (ULN).
    c) Bilirubin ≤ 1.5 x ULN, AST or ALT ≤ 2.5 x ULN.
9. Have signed written informed consent

3.3.2 Exclusion Criteria

1. Participants who fail to meet the above criteria.
2. Prior therapy for head and neck cancer is allowed, and the number of treatments is not limited. However, any systemic therapy should have been completed at least 30 days prior to study enrollment. Any radiation to the head and neck should have been completed at least 30 days prior to study enrollment. Palliative radiation outside of the head and neck does not require a washout.
3. Pregnancy or breastfeeding. Women (patients or partners of male patients) of childbearing potential (WOCBP) must practice acceptable methods of birth control to prevent pregnancy. All WOCBP MUST have a negative pregnancy test within 4 weeks prior to registration, and this must be repeated within 72 hours prior to first receiving ruxolitinib. If the pregnancy test is positive, the patient must not receive ruxolitinib and must not be enrolled in the study.
4. Any unresolved chronic toxicity ≥ grade 2 from previous anticancer therapy (except alopecia and anemia), according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE).
5. Current active infection requiring systemic antibiotic or antifungal therapy.
6. Acute hepatitis or known HIV.
7. Treatment with a non-approved or investigational drug within 30 days prior to Day 1 of study treatment.
8. New York Heart Association (NYHA) Class III or IV heart disease.
9. History of thromboembolic event or other condition currently requiring anticoagulation with warfarin (Coumadin). Patients who are treated with low molecular weight heparin or fondaparinux are eligible.
10. History of significant bleeding disorder unrelated to cancer, including: diagnosed congenital bleeding disorders (e.g., von Willebrand’s disease, diagnosed acquired bleeding disorder within one year (e.g., acquired anti-factor VIII antibodies, or ongoing or recent (≤ 3 months) significant gastrointestinal bleeding

11. Concomitant Medications, any of the following should be considered for exclusion: Strong CYP3A4 inhibitors: (Patients must discontinue drug 7 days prior to starting ruxolitinib), including but not limited to boceprevir, clarithromycin, conivaptam, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, or voriconazole. In addition, patients will be instructed to avoid grapefruit or grapefruit juice, starfruit, or Seville oranges.
12. Prisoners or participants who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness.

3.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment will only be during the pre-operative window of 14-21 days or up to 28 days for delays in planned surgery or until:

- Progressive disease at any time
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the Investigator, indicates that continued treatment with study therapy is not in the best interest of the participant
- Excessive toxicity
- Withdrawal of informed consent (participant's decision to withdraw for any reason)
- Pregnancy
    - All women of childbearing potential should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.
    - The Investigator must immediately notify Incyte in the event of a confirmed pregnancy in a patient participating in the study.
    - Study Closure or termination
    - Noncompliance

3.5 Duration of Follow Up

Patients will be followed for up to 12 weeks post-operation, as deemed clinically necessary or removal from study, or until death, whichever occurs first. Patients removed from study for unacceptable treatment related adverse event(s) will be followed until resolution or stabilization of all treatment related adverse events to Grade 2 or lower.

3.5.1 Procedures for discontinuation

Participants who discontinue should, if possible, be seen and assessed by an investigator(s). The reason for withdrawal and the date of withdrawal must be documented. If possible, any diary cards and investigational products should be returned by the participant as soon as possible after study discontinuation. If patient had screening tissue samples obtained, has taken at least 14 days of drug, and has undergone response assessment, they are evaluable.

If the reason for withdrawal from the trial is the death of the participant, the two options for categorizing withdrawal are either disease recurrence or an adverse event (AE; more than one

AE may be documented as a reason for withdrawal). Only one event will be captured as the cause of death.

Note: death is an outcome and not an AE. All deaths that occur within the trial period or within 30 days after administration of the last dose of trial drug must be reported to Incyte primarily for the purposes of serious adverse event (SAE) reporting; however, deaths due unequivocally to progression are not SAEs.

3.6 Study Timeline

3.6.1 Primary Completion

The study will reach primary completion 24 months from the time the study opens to accrual.

3.6.2 Study Completion

The study will reach completion 36 months from the time the study opens to accrual.

4 Study Drugs

4.1 Description, Supply and Storage of Investigational Drugs

4.1.1 Ruxolitinib (JAKAFI®, INCB018424)

Ruxolitinib is dosed orally, every morning and every evening, and can be administered with or without food. If a dose is missed, the patient should not take an additional dose, but should take the next scheduled prescribed dose. In the case of emesis, the patient should not take an additional dose, but should take the next scheduled prescribed dose. For patients unable to ingest tablets, ruxolitinib can be administered through a nasogastric or percutaneous gastrostomy tube (8 French or greater) as follows:

- Suspend three or four 5 mg tablets (dose dependent according to platelet count, as described under “Study drug treatment” in section 5.2) in approximately 40 mL of water with stirring for approximately 10 minutes.
- Within 6 hours after the tablets have dispersed, the suspension can be administered through the feeding tube using an appropriate syringe.

The tube should be rinsed with approximately 75 mL of water. The effect of tube feeding preparations on ruxolitinib exposure during administration through a nasogastric tube has not been evaluated.

Participants will be asked to indicate on a drug diary (see Appendix 4) when doses of study drug are taken in order to monitor for compliance. Participants may also use the drug diary for notations on side effects and other treatment related events. The Investigational Pharmacy will maintain a drug accountability log to record ruxolitinib received, dispensed and/or destroyed. Unused drugs will be destroyed locally and will not be returned.

NOTE: for current information, please see the most recent version of the Investigator Brochure

Classification

Ruxolitinib is a Class I molecule under the Biopharmaceutical Classification System, with high permeability, high solubility and rapid dissolution characteristics. In clinical studies, ruxolitinib is rapidly absorbed after oral administration with maximal plasma concentration (C_{max}) achieved approximately 1 hour post-dose

Mechanism of Action
Ruxolitinib (INCB018424 phosphate, INC424, ruxolitinib phosphate) represents a novel, potent, and selective inhibitor of JAK1 (Janus kinase 1) (inhibition concentration 50% [IC50]=3.3 ± 1.2 nM) and JAK2 (IC50=2.8 ± 1.2 nM) with modest to marked selectivity against TYK2 (tyrosine kinase 2) (IC50=19 ± 3.2 nM) and JAK3 (IC50=428 ± 243 nM), respectively. Ruxolitinib interferes with the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function.

Metabolism
Ruxolitinib was eliminated predominantly by oxidative metabolism (leading to hydroxylated metabolites M7, M8, M14 and M18 and keto metabolites M14 and M18 (Figure 4-1), followed in some cases by limited subsequent glucuronidation in humans and animal species

Contraindications
Hypersensitivity to the active substance or any of the excipients.

Potential Drug-Drug Interactions
Ruxolitinib is primarily protein bound (97%) and is metabolized by CYP3A4 in the liver. Significant drug-drug interaction may occur when ruxolitinib is administered with inducers or inhibitors of CYP3A4. Concomitant administration of ruxolitinib with strong inhibitors of CYP3A4 such as ketoconazole may lead to significant increases in C_{max}, AUC, and half-life, thus such patients should not be included on study. No dose adjustment is recommended when ruxolitinib is administered with mild inhibitors or inducers of CYP3A4, although these patients should be carefully monitored. Grapefruit juice is also a potent CYP3A4 inhibitor, thus the consumption of grapefruit juice should be avoided during ruxolitinib treatment.

Warnings and Precautions
Based on clinical experience to date in myelofibrosis, the following adverse effects may be associated with ruxolitinib: thrombocytopenia, anemia, neutropenia, bruising dizziness, headache, urinary tract infection, weight gain, flatulence, and herpes zoster. Thrombocytopenia, anemia, and neutropenia are dose-related effects, which occur at a median of 8 weeks after initiation of ruxolitinib, and in studies of longer duration have been generally treated by withholding ruxolitinib. Occasionally patients with anemia may require a transfusion. Patients taking ruxolitinib may be at risk for infection and should be monitored for this. The incidence of clinically significant hematologic toxicity is expected to be very low in the current study population, due to the short ruxolitinib exposure, as well as the absence of a primary bone marrow malignancy predisposing to hematologic toxicity.

Some people who take ruxolitinib have developed certain types of non-melanoma skin cancers.

Availability
All tablets are oval and white with “INCY” on one side and “25” on the other. Additional information regarding ruxolitinib can be found in the package insert.

Storage and handling

In addition to the active ingredient, each ruxolitinib tablet contains microcrystalline cellulose, lactose monohydrate, magnesium stearate, colloidal silicone dioxide, sodium starch glycolate, povidone, and hydroxypropyl cellulose. Ruxolitinib will be supplied as 5 mg tablets in bottles containing 60 tablets each. Labels contain, at a minimum, the following information: product name, tablet strength, batch number, directions for use, storage conditions, and appropriate caution statements. Ruxolitinib tablets should be stored in a secure area at 25˚C (77ºF); excursions permitted between 15˚-30˚C (59˚-86˚F).

Side Effects

Complete and updated adverse event information is available in the Investigational Drug Brochure and/or product package insert.

4.2 Drug Accountability

The Investigational Pharmacist will manage drug accountability records. Damaged supplies will be destroyed at the study site and adequate records of the damaged supplies will be kept at the site. Study investigators will be responsible for drug accountability.

4.3 Drug Ordering

Ruxolitinib will be supplied by Incyte Pharmaceuticals.

4.4 Packaging and Labeling of Study Drugs

Drugs will be packaged and labeled per UCSF institutional standards, adhering to applicable local and federal laws.

5 Treatment Plan

5.1 Dosage and Administration

Treatment will be administered on an outpatient basis.

Table 5.1 Regimen Description

Study Drug	Dose	Route	Schedule	Cycle Length
Ruxolitinib	20mg or 15mg dependent on baseline platelet count	PO	BID	Pre-operative window (14-21 days) or up to 28 days for delayed surgery

5.2 Dose De-escalation Rule

A continuous monitoring rule for safety will be instituted, to guard against excess toxicity from pre-operative treatment with ruxolitinib at the FDA-indicated starting dose for patients with myelofibrosis (20 mg bid for patients with a platelet count ≥ 200,000 or 15 mg bid for patients with a platelet count ≥ 150,000 and < 200,000). See section 8.3.3 Safety Lead-In

Table 5.2 below describes the number of toxicity events per number of treated patients required to trigger the ruxolitinib de-escalation rule. Table 8 also shows the posterior probability that the rate exceeds 33%, and the binomial probability associated with the decision for an assumed

33% discontinuation rate. The prior probability has a beta distributions with parameters a = 1 and b = 9, assumes a 10% mean with an approximate 80% mid-range of .0 to .22.

Table 5.2 Boundaries for the De-Escalation Rule

Participants	Treatment-Related Discontinuations	PP(π > 33%)*	Pr(X ≥ r|p = .33)
6	5	.629	.017
8	6	.685	.019
11	7	.673	.037
14	8	.664	.054
17	9	.657	.071
20	10	.650	.087
23	11	.645	.101
27	12	.594	.145
30	13	.592	.156
33	14	.590	.167

*π is the discontinuation rate. The minimum acceptable upper bound of a treatment-related discontinuation is 33%. PP(π > 33%) is the posterior probability that the discontinuation rate exceeds this 33% upper bound. This posterior probability of discontinuation is calculated from the prior distribution, the number of participants treated and the observed number of treatment-related discontinuations.

5.3 Procedures in Case of Overdose

There are no known antidotes for ruxolitinib. The treatment of AEs associated with overdose should be supportive for the underlying adverse symptoms.

Single doses up to 200 mg have been given with acceptable acute toxicity. Doses of study treatment in excess of that specified (20 mg twice daily) in the clinical study protocol are considered to be an overdose. Overdose, with or without associated symptoms, should be handled in the same way as a SAE. Signs or symptoms of an overdose that meet the criteria of SAE should be reported as a SAE in the appropriate manner and be documented as clinical sequelae to an overdose.

5.4 Procedures in Case of Pregnancy

Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive treatment. All reports of congenital abnormalities/birth defects are SAEs. Any SAE experienced during pregnancy must be reported on the SAE Report Form. Elective terminations for medical reasons, and any serious complications of pregnancy (including spontaneous miscarriage) should be reported as SAEs. Elective abortions without complications should not be handled as SAEs.

The time period for collecting information on the occurrence of a pregnancy is from first administration of study treatment up to and including the follow up period. The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality/birth defects) must be followed up and documented even if the participant was discontinued from the study.

5.5 Dose Modifications and Dosing Delays

The following dose modification rules will be used with respect to potential toxicity. Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0).

Ruxolitinib is approved for use in myelofibrosis at the dose of 20 mg po bid when the baseline platelet count > 200,000, 15 mg po bid when the baseline platelet count is > 100,000 but ≤ 200,000, and 5 mg bid when the baseline platelet count is > 50,000 but ≤ 100,000. Ruxolitinib is approved in polycythemia vera at the dose of 10 mg po bid.

Dose reductions will not be allowed. Treatment delays up to 1 week are permitted as follows:

- If participants experience any grade 3-4 ruxolitinib-related non-hematologic toxicities in the neo-adjuvant window, the study drug will be discontinued and they will be removed from the study. (Exception: Patients with asymptomatic grade 3 electrolyte abnormalities manageable with repletion) Patients may also be removed from study for intolerable non-hematologic toxicities of any grade, which persist despite optimal medical management.
- If participants experience grade ≥ 3 thrombocytopenia or neutropenia, ruxolitinib will be discontinued and they will be removed from the study.
- Grade 3 anemia does not require discontinuation from protocol. Patients may be transfused per discretion of the treating physicians.
- For mild or moderate (grade 1 or 2) side effects that are difficult to tolerate, treatment may be held for up to 1 week. Treatment may be resumed at the same dose, if there is improvement by at least one grade. Dose delays more than 1 week will not be permitted. If treatment is restarted, study physicians should aim for at least 3 days of ruxolitinib exposure prior to planned surgery.

In two Phase 3 clinical studies of ruxolitinib in myelofibrosis, the median time to onset of first CTCAE grade two or higher anemia was 6 weeks, and approximately 8 weeks until onset of grade 3 or 4 thrombocytopenia. In nearly all cases, ruxolitinib-induced anemia and thrombocytopenia could be reversed with dose reduction or interruption; occasionally red blood cell or platelet transfusions were required. Thus, given the short 2-3 weeks of treatment during this study, it is expected that few, if any, participants will develop hematologic abnormalities. Moreover, participants in the current study do not have a primary bone marrow malignancy which is the target of ruxolitinib and thus predisposes to hematologic toxicity. Any participants that experience anemia or thrombocytopenia may be transfused if deemed necessary by the treating physician.

Although clinically significant hematologic and non-hematologic toxicity is unexpected during the 14-21 days of protocol treatment in patients with operable HNSCC, a continuous monitoring rule for excess toxicity will be instituted. Please see Section 5.6 below.

5.6 Monitoring and Toxicity Management

Each patient receiving ruxolitinib will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and spontaneous reports of adverse events reported to the investigator by patients.

Each patient will be assessed periodically for the development of any toxicity as outlined in Section 6 Study Procedures and Observations. Toxicity will be assessed according to the NCI

CTCAE v4.0. Dose adjustments will be made according to the system showing the greatest degree of toxicity.

We will monitor for the following anticipated adverse events:

Anemia
In the two Phase 3 clinical studies, median time to onset of first CTCAE Grade 2 or higher anemia was approximately 6 weeks. One patient (0.3%) discontinued treatment because of anemia. In patients receiving ruxolitinib, mean decreases in hemoglobin reached a nadir of approximately 1.5 to 2.0 g/dL below baseline after 8 to 12 weeks of therapy and then gradually recovered to reach a new steady state that was approximately 1.0 g/dL below baseline. This pattern was observed in patients regardless of whether they had received transfusions during therapy.

In the randomized, placebo-controlled study, 60% of patients treated with Jakafi and 38% of patients receiving placebo received red blood cell transfusions during randomized treatment. Among transfused patients, the median number of units transfused per month was 1.2 in patients treated with ruxolitinib and 1.7 in placebo treated patients.

Thrombocytopenia
In the two Phase 3 clinical studies, in patients who developed Grade 3 or 4 thrombocytopenia, the median time to onset was approximately 8 weeks. Thrombocytopenia was generally reversible with dose reduction or dose interruption. The median time to recovery of platelet counts above 50 X 10^9/L was 14 days. Platelet transfusions were administered to 4.7% of patients receiving ruxolitinib and to 4.0% of patients receiving control regimens. Discontinuation of treatment because of thrombocytopenia occurred in 0.7% of patients receiving ruxolitinib and 0.9% of patients receiving control regimens. Patients with a platelet count of 100 X 10^9/L to 200 X 10^9/L before starting ruxolitinib had a higher frequency of Grade 3 or 4 thrombocytopenia compared to patients with a platelet count greater than 200 X 10^9/L (16.5% versus 7.2%).

Neutropenia
In the two Phase 3 clinical studies, 1.0% of patients reduced or stopped ruxolitinib because of neutropenia.

Table 5.6 provides the frequency and severity of clinical hematology abnormalities reported for patients receiving treatment with Jakafi or placebo in the placebo-controlled study.

Table 5.6 Worst Hematology Laboratory Abnormalities in the Placebo-controlled Study[a]

	Jakafi (N=155)			Placebo (N=151)		
Laboratory Parameter	**All Grades[b] (%)**	**Grade 3 (%)**	**Grade 4 (%)**	**All Grades (%)**	**Grade 3 (%)**	**Grade 4 (%)**
Thrombocytopenia	69.7	9.0	3.9	30.5	1.3	0
Anemia	96.1	34.2	11.0	86.8	15.9	3.3
Neutropenia	18.7	5.2	1.9	4.0	0.7	1.3

a Presented values are worst Grade values regardless of baseline

b National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0

Additional Data from the Placebo-controlled Study

25.2% of patients treated with ruxolitinib and 7.3% of patients treated with placebo developed newly occurring or worsening Grade 1 abnormalities in alanine transaminase (ALT). The incidence of greater than or equal to Grade 2 elevations was 1.9% for ruxolitinib with 1.3% Grade 3 and no Grade 4 ALT elevations.

17.4% of patients treated with ruxolitinib and 6.0% of patients treated with placebo developed newly occurring or worsening Grade 1 abnormalities in aspartate transaminase (AST). The incidence of Grade 2 AST elevations was 0.6% for ruxolitinib with no Grade 3 or 4 AST elevations.

16.8% of patients treated with ruxolitinib and 0.7% of patients treated with placebo developed newly occurring or worsening Grade 1 elevations in cholesterol. The incidence of Grade 2 cholesterol elevations was 0.6% for ruxolitinib with no Grade 3 or 4 cholesterol elevations.

6 Study Procedures and Observations

The study-specific procedures and assessments are outlined in the Study Calendar – Section 6.1.

Screening assessments must be performed within 28 days prior to the first dose of investigational product. Any results falling outside of the reference ranges may be repeated at the discretion of the investigator.

All on-study visit procedures are allowed **a window of ±5 days** unless otherwise noted. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

6.1 Study Calendar

Table 6.1 Schedule of Study Procedures and Assessments

Study Procedures	Screening	Treatment Period			Surgery (between 14-28 days of ruxolitinib start)[g]	4-week post-op visit	Final study visit (12-week post-op)
		Days 1 thru 14 to 21	Days 22 thru 28 (if surgery is delayed)	Pre-surgery visit (within 5 days prior to surgery)			
Informed Consent	X						
History and Physical Examination[a]	X			X		X	X
Vital Signs, Weight	X			X		X	X
ECOG-PS	X			X		X	X
Pregnancy Test[b]	X						
EKG[c]	X						
Clinical Blood Tests[d]	X			X		X	X
Cross-sectional imaging study[e]	X			X			
Research Blood Collection[h]	X			X			
Ruxolitinib[f]		X	X	X (take until morning of surgery)			
Surgery[g]					X		
Tumor sample[i]	X				X		
Archival tissue collection	X						

a. History and physical examination within 8 weeks prior to study entry. This should include surgical assessment of the primary tumor, including fiberoptic nasopharyngoscopy or examination under anesthesia as indicated per standard of care, and as determined by the treating surgeon. Patients will be reevaluated within 5 days prior to scheduled surgery. Toxicity and compliance as evidenced by pill count and patient diaries will be assessed.
b. Pre-menopausal women of childbearing potential (WOCBP) must have a negative urine or serum pregnancy test within 4 weeks of registration. A negative pregnancy test must then be documented within 72 hours of Day 1 of ruxolitinib treatment.
c. The standard of care EKG performed for pre-surgical evaluation should be used. EKGs performed within 12 weeks prior to study entry may be substituted, provided the patient has experienced no interval cardiac event.

d. Clinical blood tests include CBC with differential (to include total white blood cell count, absolute neutrophil count, hemoglobin, hematocrit and platelets), serum chemistries (to include sodium, potassium, chloride, HCO_3, BUN, creatinine, glucose, magnesium and calcium), liver function tests (to include total bilirubin, AST, ALT, total protein, albumin and alkaline phosphatase), coagulation studies (PT/INR and PTT), and C-reactive protein (CRP). Clinical blood tests will be repeated once within 5 days prior to surgery, and at the 4-week and 12-week post-operative visits if clinically indicated according to the investigator. **Coagulation studies are not required at the 4 week or 12 week post-surgical visits.**
e. Within 6 weeks of study entry (within 4 weeks preferred), and again within 5 days prior to planned surgery, patients will undergo cross-sectional radiological evaluation using diagnostic contrasted CT, PET/CT (with diagnostic contrasted CT), or MRI of regions with radiologically or clinically identifiable tumor. The minimum cross-sectional evaluation is a diagnostic, contrasted computed tomography (CT) scan of the neck. PET/CT is the preferred modality, **provided it includes a diagnostic, contrasted CT scan of the neck** – however is not mandatory. For patients with severe allergy to iodinated contrast dye despite premedication, or in the case of physician preference, neck MRI may be substituted. The pre- and post-treatment imaging modality should be the same, i.e. if MRI was used at study entry, it should be used at the post-treatment, pre-surgical assessment. **NOTE**: in the event that a patient lacks a CT-measurable primary tumor or metastatic lymph node, measurable disease may also be established by caliper measurement of an oral cavity or oropharyngeal tumor (≥ 1 centimeter). In this case, caliper measurement should be repeated within 5 days prior to planned surgery.
f. The first day of ruxolitinib treatment will be considered Day 1. Ruxolitinib will be administered at 20 mg po bid, for 1421 days, and will be discontinued on the day of surgical resection (following the morning dose). If surgery is delayed, the study drug may be continued until surgery, for a maximum of 28 days. The interval between the last dose of ruxolitinib and surgery will be 2-24 hours.
g. Surgery will be scheduled during days 14-21 of ruxolitinib treatment. For logistical reasons, surgery may be delayed by up to 7 days. Postoperative complications, hospital days, and ICU days will be noted for each patient.
h. Research blood samples will be obtained at screening and within the 5 days prior to scheduled surgery. One (1) purple top EDTA tube will be collected at each time point. Please refer to the laboratory SOPs for processing instructions.
i. Archival tissue if available. If no archival tissue is available participants must undergo tissue biopsy at baseline. Research biopsies obtained at screening during planned surgery for resection of the tumor will be divided into fresh, fresh-frozen and paraffin specimens.
j. Patients will be seen for a standard 4 week post-operative visit (+/- 2 weeks), and again for a standard 12-week post-operative visit (+/- 4 weeks). Clinical blood tests will be obtained as deemed clinically necessary. Thereafter, patients will be followed off study per standard of care.

6.2 Participant Registration

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF will be given to the participant and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS). The system is password protected and meets HIPAA requirements.

Each participating site is responsible for OnCore® registration of study participants consented at the site.

6.3 Schedule of Procedures and Observations

6.3.1 Screening Period

Pretreatment or Screening Evaluations (Baseline, within 4 weeks prior to study registration unless otherwise specified)

Required screening evaluations include:

- Tumor tissue sample:
    - Patients who have had research tissue procured under an omnibus tissue consent, who are determined to have sufficient fresh, fresh-frozen, and paraffin tissue for biomarker analysis, may substitute the archived tissue and do not need to undergo screening research biopsy provided no interval anti-neoplastic therapy was given.
    - Baseline research biopsy: If no archival tissue is available participants must undergo tissue biopsy at baseline (see below):
        - Examination under anesthesia (EUA) with triple endoscopy is strongly recommended, if not previously performed and/or if an appropriate standard of care for surgical staging. EUA may include direct laryngoscopy, bronchoscopy and esophagoscopy in order to fully evaluate the extent of primary tumor and rule out evidence of a second primary aerodigestive tract tumor. A thorough description of the anatomical extent of the head and neck primary tumor will be detailed by the surgeon in the EUA operative report. Biopsy of the primary tumor for diagnosis, if not already done, will be performed during the EUA according to standard pathologic procedures. Research biopsy of the primary tumor for biomarker studies during the EUA is required if no archival tissue is available. Alternately, patients for whom an in-office biopsy with local anesthesia is feasible may have the baseline research biopsy performed in the outpatient office of the otolaryngologist or head and neck surgeon.
    - Tissue for biomarker analysis will be processed according to procedures described in Appendix 5.

- Blood collection. The patient is required to undergo blood collection for biomarker assessment. For logistical purposes, research labs may be drawn simultaneous with blood labs required for standard clinical management.
    - Blood for biomarker analysis will be processed according to procedures described in Appendix 5.
- History and physical examination, to include surgical and medical evaluation, within 8 weeks prior to study registration, including a careful description of the location and extent of the primary lesion and nodal spread. Fiberoptic nasopharyngoscopy/laryngoscopy is recommended, unless fiberoptic exam is deemed unnecessary by the treating surgeon.
- Vital signs, weight, height within 8 weeks prior to study registration
- ECOG Performance Status within 8 weeks prior to study registration
- Laboratory studies including CBC with differential (to include total white blood cell count, absolute neutrophil count, hemoglobin, hematocrit and platelets), serum chemistries (to include sodium, potassium, chloride, carbon dioxide, BUN, creatinine, glucose, magnesium and calcium), liver function tests (LFTs; to include total bilirubin, AST, ALT, total protein, albumin and alkaline phosphatase), coagulation studies (PT/INR and PTT), and C-reactive protein (CRP).
- Negative serum pregnancy test within 4 weeks of study registration in WOCBP. Note, the clinical research coordinator or treating investigator will document an additional negative pregnancy test within 72 hours of starting ruxolitinib treatment.
- Cross-sectional radiologic evaluation within 6 weeks of study registration (within 4 weeks is preferred). The minimum cross-sectional evaluation is a diagnostic, contrasted computed tomography (CT) scan of the neck. PET/CT is the preferred modality, **provided it includes a diagnostic, contrasted CT scan of the neck** – however is not mandatory. For patients with severe allergy to iodinated contrast dye despite premedication, or in the case of physician preference, neck MRI may be substituted. If MRI is used for baseline imaging, it should also be used for response assessment (post-treatment, pre-surgical imaging). This is standard of care for baseline assessment. The post treatment assessment is research.
- EKG. The standard of care EKG performed for pre-surgical evaluation should be used. EKGs performed within 12 weeks prior to study registration may be substituted, provided the patient has experienced no interval cardiac event.

6.3.2 **Treatment Period**

Days 1-28; **NOTE** ruxolitinib treatment is planned for 14-21 days, however may be administered up to 28 days if required for logistical/scheduling purposes.

6.3.2.1 **Day 1**

Ruxolitinib will be administered at the FDA-approved dose in myelofibrosis, as determined by baseline platelet count: 1) Patients with a platelet count ≥ 200,000 will take 20 mg bid (four 5mg tablets in the morning and four 5mg tablets in the evening); 2) participants with a platelet count ≥ 150,000 and < 200,000 will take 15 mg bid (three 5 mg tablets in the morning and three 5 mg tablets in the evening). Ruxolitinib will be discontinued on the day of surgical resection (following the morning dose). If surgery is delayed, the study drug may be continued until surgery, for a

maximum of 28 days. Fourteen days is the minimum required treatment for a patient to be considered evaluable. The interval between the last dose of ruxolitinib and surgery will be 2-24 hours.

Ruxolitinib will be dispensed as 5mg tablets. Participants with a platelet count ≥ 200,000 will take 20 mg bid (four 5mg tablets in the morning and four 5mg tablets in the evening). Participants with a platelet count ≥ 150,000 and < 200,000 will take 15 mg bid (three 5 mg tablets in the morning and three 5 mg tablets in the evening).

Participants will be asked to indicate on a drug diary (see Appendix 4) when doses of study drug are taken in order to monitor for compliance. Participants may also use the drug diary for notations on side effects and other treatment related events.

6.3.2.2 **Day 9-28 (inclusive of day of surgery)**

Post-treatment, pre-surgical
NOTE: all efforts should be made to schedule these evaluations on the day prior to or the day of surgery, however a 5 day window is provided for logistical purposes.

- *History and Physical Examination (Post-treatment, Pre-operative):* Participants will be evaluated by history and physical examination by the treating physician within 5 days prior to planned surgery (including vital signs, weight, performance status). This visit will also include toxicity and compliance assessment. Compliance as evidenced by pill count and patient drug diary will be assessed. For logistical convenience, this visit may be scheduled in conjunction with blood collection and imaging as specified below. NOTE: Participants will continue to take ruxolitinib until the morning of planned surgery. If surgery is delayed, participants will continue to take ruxolitinib and this pre-operative history and physical examination does not need to be repeated.
- *Blood Collection (Clinical and Research Blood):* Participants will undergo repeat CBC with differential, serum chemistries, LFTs, coagulation studies and C-reactive protein within 5 days prior to planned surgery. Research blood will be drawn simultaneously with these standard pre-operative labs. NOTE: Participants will continue to take ruxolitinib until the morning of planned surgery. If surgery is delayed, participants will continue to take ruxolitinib and these pre-operative blood tests do not need to be repeated.
- *Cross-sectional Imaging for Tumor Measurements (Research)*: Participants will undergo a repeat diagnostic CT scan of the neck with IV contrast within 5 days prior to planned surgery. If a neck MRI was performed in lieu of CT scan at baseline, then a MRI should be substituted at response assessment. NOTE: Participants will continue to take ruxolitinib until the morning of planned surgery. If surgery is delayed, participants will continue to take ruxolitinib and this pre-operative imaging does not need to be repeated.

  NOTE: in the event that a patient lacks a CT-measurable primary tumor or metastatic lymph node, measurable disease may be established by caliper measurement of an oral cavity or oropharyngeal tumor (≥ 1 centimeter). In this case, caliper measurement should be repeated and documented within 5 days prior to planned surgery, as a substitute for cross-sectional imaging.

6.3.2.3 **Surgery (Occurring Between Days 14-28, Inclusive)**

The nature of complete resection of the primary head and neck tumor, type of reconstruction, and levels of nodes to be dissected will be determined by the treating surgeon. Prior to surgery, dental evaluation is recommended, to allow for any necessary dental extractions to be planned in conjunction with surgery. Consultations with a Nutritionist and Speech and Language Pathologist are strongly recommended prior to surgery and as ongoing support. Placement of a nasogastric (e.g. Dobhoff) or gastrostomy feeding tube is at the discretion of the participant and the study physicians. Following 14-21 days (+ 7 days if required for logistical and scheduling purposes) of treatment prior to participant's scheduled surgery, a surgeon will resect the participant's tumor (days 14-28). Part of the tumor specimen will be sent to the research laboratory for mandatory biomarker analyses. If surgery is unexpectedly cancelled, the patient will be removed from the study unless the primary tumor is accessible for in-office biopsy in the judgment of the surgeon-investigator, in which case a tumor biopsy may be substituted for the operative specimen. Tissue collection procedures are described in Section 9.0.

6.3.2.4 **Post-operative therapy**

After surgery, participants may receive adjuvant radiation or radiation plus chemotherapy in accordance with appropriate standards, as determined by the participant's treating physicians.

6.3.3 **End-of-Treatment/Final Study Visit Procedures**

A standard history and physical examination and blood tests as clinically indicated (CBC, chemistries, liver function tests in accordance with investigator judgment) will be performed 4 weeks (+/- 2 week) and 12 weeks (+/- 4 weeks) post-surgery. Additional non-protocol visits may occur during the 12-week post-operative period as deemed clinically necessary. Patients will be referred for adjuvant chemotherapy and radiation per standard of care, based upon pathologic findings. Once the first 12 weeks of follow-up have been completed, study follow-up will be discontinued and participants will continue standard treatment and surveillance in accordance with national guidelines.

The standard post-operative visit which occurs 12 weeks post-op (+/- 4 weeks) will be considered the "final study visit."

Post-Therapy Radiologic Evaluation
Within 6 weeks prior to registration (within 4 weeks preferred), and again within the 5 days prior to planned surgery, patients will undergo cross-sectional radiological evaluation using diagnostic contrasted CT, PET/CT (with diagnostic contrasted CT), or MRI of regions with radiologically or clinically identifiable tumor. The minimum cross-sectional evaluation is a diagnostic, contrasted computed tomography (CT) scan of the neck. PET/CT is the preferred modality, **provided it includes a diagnostic, contrasted CT scan of the neck** – however is not mandatory. For patients with severe allergy to iodinated contrast dye despite premedication, or in the case of physician preference, neck MRI may be substituted. The pre- and post-treatment imaging modality should be the same, i.e. if MRI was used at study entry, it should be used at the post-treatment, pre-surgical assessment. Modified Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1)[37] criteria will be used to establish target lesions.

NOTE: in the event that a patient lacks a CT-measurable primary tumor or metastatic lymph node, measurable disease may also be established by caliper measurement of an oral cavity or

oropharyngeal tumor (≥ 1 centimeter). In this case, caliper measurement should be repeated within 5 days prior to planned surgery.

6.4 Usage of Concurrent/Concomitant Medications

6.4.1 Dietary Restrictions

Patients will be instructed to avoid grapefruit or grapefruit juice, starfruit, or Seville oranges.

6.4.2 Prohibited Medications

Strong CYP3A4 inhibitors. For eligibility, patients must discontinue drug 7 days prior to starting ruxolitinib, including but not limited to boceprevir, clarithromycin, conivaptam, indinavir, itraconazle, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, or voriconazole.

7 Reporting and Documentation of Results

7.1 Evaluation of Efficacy: Antitumor Effect – Solid Tumors

Response and progression in this study will be evaluated using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [JNCI 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria (or International Workshop on Chronic Lymphocytic Leukemia [IWCLL]).

7.1.1 Definitions

Evaluable for toxicity

All patients will be evaluable for toxicity from the time of their first treatment with the study drug.

Evaluable for objective response

Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

7.1.2 Disease Parameters

Measurable disease

Measurable disease is defined as lesions (or tumors) that can be accurately measured in at least one dimension (longest diameter to be recorded) with a minimum size of 10mm by CT scan (irrespective of scanner type) and MRI (no less than double the slice thickness and a minimum of 10mm), 10mm caliper measurement by clinical exam (when superficial), and/or 20mm by chest X-ray (if clearly defined and surrounded by aerated lung).

All tumor measurements will be recorded in millimeters or decimal fractions of centimeters. Previously irradiated lesions are considered non-measurable except in cases of documented progression of the lesion since the completion of radiation therapy.

Target lesions

All measurable lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions will be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. It is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. “multiple enlarged pelvic lymph nodes” or “multiple liver metastases”). Bone lesions may be measureable if ≥ 1 cm on MRI. Measurements of these lesions are not required, but the presence or absence of each will be noted throughout follow-up.

Non-measurable disease (Tumor Markers)

Non-measurable disease is all other lesions (or sites of disease), including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm using spiral CT scan). Leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques are all non-measurable. (e.g., PSA, CA-125, CA19-9, CEA)

7.1.3 Methods for Evaluation of Measurable Disease

All measurements will be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 28 days before the beginning of the treatment.

The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

7.1.4 Response Criteria

Complete Response (CR)

Disappearance of all target lesions, determined by two separate observations conducted not less than 4 weeks apart. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (the sum may not be “0” if there are target nodes). There can be no appearance of new lesions.

Partial Response (PR)
At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. There can be no appearance of new lesions.

Progressive Disease (PD)
At least a 20% increase in the sum of the SLD of target lesions, taking as reference the smallest sum SLD recorded since the treatment started and minimum 5 mm increase over the nadir, or the appearance of one or more new lesions.

Stable Disease (SD)
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

Evaluation of Non-Target Lesions

Complete Response (CR)
Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Incomplete Response/Stable Disease (SD)
Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD)
Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Evaluation of Best Overall Response
The best overall response is the best response recorded from the start of the treatment until disease progression.

Evaluation of Target Lesions

Complete Response (CR)
Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR)
At least a 30% decrease in the sum of target lesion diameters (longest diameter of non-nodal lesions; short axis diameter of the target lymph nodes), /recurrence (taking as reference the *baseline sum diameter*.

Progressive Disease (PD)
At least a 20% increase in the sum of target lesion diameters (longest diameter of non-nodal lesions; short axis diameter of the target lymph nodes), taking as reference for progressive disease the smallest *sum diameter* measurements recorded since the baseline sum diameter measurements. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. Note: the appearance of one or more new lesions is also considered progressions. The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 2.1 Response Criteria

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category Also Requires
CR	CR	No	CR	> 4 weeks confirmation
CR	Non-CR/ Non-PD	No	PR	> 4 weeks confirmation
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	documented at least once > 4 weeks from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression

Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression.

7.2 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE v4.0 for reporting of non-hematologic adverse events and modified criteria for hematologic adverse events, see Appendix 2.

The Principal Investigator at the UCSF Coordinating Center will hold the role of Study Chair. The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating sites.

7.3 Definitions of Adverse Events

7.3.1 Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event (can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

7.3.2 Adverse Reaction

An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

7.3.2.1 Suspected

A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting,

"reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

7.3.2.2 **Unexpected**

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

7.3.2.3 **Serious**

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

1. In the event of a serious adverse event, the PI, the institutional review board (per institutional reporting requirements), and Incyte Corporation will be notified using the FDA Form 3500 MedWatch report. All events meeting the definition of a serious adverse event should be recorded on a MedWatch 3500 Form (http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM163919.pdf) and submitted to:DSMC
2. Local IRB per institutional reporting requirements
3. FDA
4. Incyte

- In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the Event Description (section 5) of the MedWatch 3500 form:CTCAE term(s) and grade(s)
- Current status of study drug
- All interventions to address the AE (testing and result, treatment and response)
- Hospitalization and/or discharge dates
- Event relationship to study drug

7.3.2.4 **Life-threatening**

An adverse event or suspected adverse reaction is considered *life threatening* if, in the view of either the investigator or sponsor, its occurrence places the patient or participant at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

7.4 **Recording of an Adverse Event**

All grade 3 and above adverse events will be entered into OnCore®, whether or not the event is believed to be associated with use of the study drug. Data about these events and their severity will be recorded using the NCI CTCAE v4.0.

The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into OnCore® using the classification system listed below:

Relationship	Attribution	Description
Unrelated to investigational drug/intervention	Unrelated	The AE *is clearly NOT related* to the intervention
	Unlikely	The AE *is doubtfully related* to the intervention
Related to investigational drug/intervention	Possible	The AE *may be related* to the intervention
	Probable	The AE *is likely related* to the intervention
	Definite	The AE *is clearly related* to the intervention

Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE, they will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0	No AE (or within normal limits)
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated
Grade 5:	Death related to AE

7.5 Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until resolved. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the participant with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

Follow-up Reports:
Additional information may be added to a previously submitted report by adding to the original MedWatch 3500 report and submitting it as follow-up or creating supplemental summary information and submitting it as follow-up with the original MedWatch 3500 form.

7.6 Adverse Events Monitoring

All adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, as noted above.

The Investigator will assess all adverse events and determine reportability requirements to the UCSF Data and Safety Monitoring Committee (DSMC) and UCSF's Institutional Review Board, the Institutional Review Board (IRB); and, when the study is conducted under an Investigational New Drug Application (IND), to the Food and Drug Administration (FDA) if it meets the FDA reporting criteria.

All adverse events entered into OnCore® will be reviewed by the Helen Diller Family Comprehensive Cancer Center Site Committee on a weekly basis. The Site Committee will review and discuss at each weekly meeting the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

All grade(s) 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all suspected adverse reactions considered "serious" entered into OnCore®, will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks.

For a detailed description of the Data and Safety Monitoring Plan for a Multicenter Phase 2 or 3 Institutional Study at the Helen Diller Comprehensive Cancer Center please refer Appendix 2.

7.7 Expedited Reporting

Reporting to the Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee must notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail.

Reporting to UCSF Institutional Review Board (Institutional Review Board)

The Principal Investigator must report events meeting the UCSF IRB definition of “Unanticipated Problem” (UP) within 5 business days of his/her awareness of the event.

Expedited Reporting to the Food and Drug Administration

If the study is being conducted under an IND, the Sponsor-Investigator is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The Investigator must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The Sponsor-Investigator needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Unexpected
- Serious

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator’s initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

Reporting to Pharmaceutical Companies providing Study Drug

Incyte Corporation, Adverse Events Reporting (within 24 hours of learning of the SAE):

Phone: (855) 463-3463

8 Statistical Considerations and Evaluation of Results

8.1 Study Endpoints

This is a phase II open label study to identify baseline and/or pharmacodynamics biomarkers of clinical response to ruxolitinib based on quantitative change in tumor size following 14-21 days of neoadjuvant ruxolitinib in patients with operable HNSCC. Clinical response will be defined by the change of the ΔTumor size between two measurements during study windows.

8.2 Determination of Sample Size and Accrual Rate

8.2.1 Sample Size and Power Estimate

The primary objective of the study is to identify baseline and/or pharmacodynamic biomarkers that might have predictive utility to determine the clinical response to ruxolitinib. As a biomarker study, the statistical design is to accrue a sufficient number of patients and provide 80% power to test one or any of 5 key hypotheses with a shared alpha of 0.15: 1) relationship between clinical response (ΔTumor size) and baseline STAT activation (pSTAT3 expression); 2) relationship between clinical response (ΔTumor size) and change in expression of pSTAT3; 3) relationship between responders (ΔTumor size) and change in expression of cyclin D1; 4) relationship between clinical response (ΔTumor size) and baseline genetic or epigenetic PTPRT or PTPRD silencing; 5) relationship between responders (ΔTumor size) and baseline C-reactive protein. An overall alpha of .15 will be shared equally among the five primary hypotheses. 23 patients with complete biomarker data are needed to achieve 80% power for the 5 primary hypothesis and limit overall family wise error rate (FWER) to 15%. As estimated from our prior window trials, accounting for treatment compliance and quality paired tumor specimens, this will require enrollment of 45 patients. For each biomarker (baseline pSTAT3 expression, change from baseline of pSTAT3 expression, change from baseline of cycline D1 expression, baseline PTPRT or PTPRD silencing and baseline C-reactive protein), mean and standard deviation will be estimated and compared for significance between clinical responders and non-responders of ruxolitinib therapy using Wilcoxon rank tests. Our experience with our phase 0 clinical trial testing the STAT3 decoy also showed correlations between ΔKi-67 (the primary pharmacodynamic efficacy endpoint) and STAT3-target genes falling in the range of .50 to .67. Twenty-five patients will enable a two -tailed Spearman Correlation test at alpha = .03 to detect a true correlation of .56. For the expected 30% of patients with a PTPR mutation, a one-tailed Wilcoxon test will demonstrate increased sensitivity to ruxolitinib via a decrease in tumor size equal to 1.3 standard deviations with 23 patients (8 with a mutation).

8.2.2 Replacement Policy

Because this is an open label study with limited number of patients, 23 accrued eligible patients with clinical tumor assessments and biomarkers measurements at baseline and the end of study window are required for the study. Additional patients will be enrolled to replace patients without clinical tumor assessments and completed biomarker measurements for both time points at least one of the five biomarkers of interest. However, all patients will be followed and included for safety assessments.

8.2.3 Safety Lead-in

A continuous monitoring rule for safety will be instituted, to guard against excess toxicity from pre-operative treatment with ruxolitinib at the FDA-indicated starting dose for patients with myelofibrosis (20 mg bid for patients with a platelet count ≥ 200,000 or 15 mg bid for patients with a platelet count ≥ 150,000 and < 200,000). The first cohort of 6 patients will be treated at this starting dose. After enrollment of the 6th patient, we will continuously monitor the number of

patients who discontinue ruxolitinib and come off study for ruxolitinib-attributable toxicity. A qualifying toxicity must be at least possibly related to ruxolitinib, as judged by the treating investigator, and fulfill one of the following definitions:

- Grade ≥ 3 non-hematologic toxicity, with the exception of asymptomatic electrolyte abnormalities manageable with repletion.
- Intolerable non-hematologic toxicities of any grade, which persist despite optimal medical management.
- Grade ≥ 3 thrombocytopenia or neutropenia.

If the posterior probability of ≥ 33% toxicity rate exceeds 50% for patients having to discontinue window treatment due to qualifying toxicity, this would be considered unacceptable and would result in de-escalating the starting dose of ruxolitinib to 10 mg p.o. bid, the FDA-indicated starting dose in polycythemia vera, for all subsequent patients. This would be the starting dose in all patients with a qualifying platelet count for this study (≥150,000), including those with platelet count ≥ 150,000 and < 200,000 and those with platelet count > 200,000. (see section 5.2 Dose De-Escalation Rule)

8.2.4 Accrual Estimates

Accrual of 2 evaluable patients per month is expected for total enrollment period of approximately 12 months.

8.3 Interim Analyses and Stopping Rules

This is an open label biomarkers study. No interim analysis is planned.

8.4 Analyses Plans

8.4.1 Analysis Populations

Safety Population

The safety population will include all enrolled patients who receive at least one dose of ruxolitinib and have the potential to complete pharmacodynamics biomarkers assessments at baseline and the end of study treatment window. Although the risk from specimen collections for biomarker assessment is limited and low, all patients will be evaluable for toxicity and safety assessment of ruxolitinib. Patients who are determined ineligible after enrollment or who are not evaluable for safety analysis (e.g. due to withdrawal of consent or failure of specimens collection as not related to tumor progression and not treatment-related toxicity) will be replaced (see section 8.2.2 for details).

Efficacy Population

The efficacy population for the primary endpoint of 35% or greater reduction in tumor size will include all enrolled patients who have measurable disease present at baseline and have completed tumor assessment at the end of study window. Patients who are determined ineligible after enrollment or who are not evaluable for safety analysis (e.g. due to withdrawal of consent or failure of specimens collection as not related to tumor progression and not treatment-related toxicity) will be replaced (see section 8.2.2 for details).

8.4.2 **Primary Analysis (or Analysis of Primary Endpoints)**

The primary objective of the study is to identify if one or any of the pharmacodynamics biomarkers of interest have predictive utility on the clinical tumor response to neoadjuvant ruxolitinib treatment in patients with operable HNSCC. The clinical response will be based on the reduction in Δtumor size from baseline to 14-21 days of ruxolitinib treatment:

(1) Primary analysis using Spearman correlation between baseline pSTAT3 expression and %Change in tumor size will also be performed. As a supportive exploratory analysis, for clinical responders of patients who have achieved a 35% or greater on reduction in Δtumor size from baseline, descriptive statistics with mean, standard deviation and 95% CI will be calculated for baseline STAT activation (pSTAT3 expression) and Wilcoxon rank tests will be used to determine the significant difference on baseline pSTAT3 expression between responders and non-responders.

(2) Primary analysis using Spearman correlation between change of pSTAT3 expression and %Change in tumor size will also be performed. As a supportive exploratory analysis, for clinical responders of patients who have achieved a 35% or greater on reduction in Δtumor size from baseline, descriptive statistics with mean, standard deviation and 95% CI will be calculated for change in STAT activation (pSTAT3 expression) from baseline and Wilcoxon rank tests will be used to determine the significant difference on change of pSTAT3 expression between responders and non-responders.

(3) Primary analysis using Spearman correlation between change of cyclin D1 expression and %Change in tumor size will also be performed. As a supportive exploratory analysis, for clinical responders of patients who have achieved a 35% or greater on reduction in Δtumor size from baseline, descriptive statistics with mean, standard deviation and 95% CI will be calculated for change in cyclin D1 expression from baseline and Wilcoxon rank tests will be used to determine the significant difference on change of cyclin D1 expression between responders and non-responders.

(4) Primary analysis using Spearman correlation between baseline genetic or epigenetic PTPRT/PTPRD silencing and %Change in tumor size will also be performed. As a supportive exploratory analysis, for clinical responders of patients who have achieved a 35% or greater on reduction in Δtumor size from baseline, descriptive statistics with mean, standard deviation and 95% CI will be calculated for baseline genetic or epigenetic PTPRT/PTPRD silencing and Wilcoxon rank tests will be used to determine the significant difference on baseline genetic or epigenetic PTPRT/PTPRD silencing between responders and non-responders.

(5) Primary analysis using Spearman correlation between baseline C-reactive protein level and %Change in tumor size will also be performed. As a supportive exploratory analysis, for clinical responders of patients who have achieved a 35% or greater on reduction in Δtumor size from baseline, descriptive statistics with mean, standard deviation and 95% CI will be calculated for baseline C-reactive protein level and Wilcoxon rank tests will be used to determine the significant difference on baseline C-reactive protein level between responders and non-responders.

8.4.3 **Secondary Analysis (or Analysis of Secondary Endpoints)**

For secondary endpoints, due to the limited sample size in the study, descriptive statistics with mean and 95% confidence intervals will be used. For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, min and

maximum. For categorical variables, descriptive statistics will include counts and percentages per category. For comparison between subgroups, ANOVA and t-tests or non-parametric tests will be used when appropriate to determine the association of the efficacy endpoints and specific biomarkers. For example, descriptive statistics with mean, standard deviation and 95% CI will be calculated for the tumoral Ki-67 proliferation index and Wilcoxon rank tests will be used to determine the significant difference on the tumoral Ki-67 proliferation index between responders and non-responders. We will also performed additional analysis using Spearman correlation between the tumoral Ki-67 proliferation index and %Change in tumor size.

8.4.4 Other Analyses/Assessments

For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, min and maximum. For categorical variables, descriptive statistics will include counts and percentages per category.

Descriptive statistics with mean, standard deviation and 95% CI will be calculated for baseline JAK/STAT3 Signaling activation and Wilcoxon rank tests will be used to determine the significant difference on baseline JAK/STAT3 Signaling activation between responders and non-responders.

Descriptive statistics with mean, standard deviation and 95% CI will be calculated for baseline and change in STAT1/STAT3 activation and Wilcoxon rank tests will be used to determine the significant difference on baseline and change in STAT1/STAT3 activation between responders and non-responders.

Descriptive statistics with mean, standard deviation and 95% CI will be calculated for baseline SHP-2 overexpression and Wilcoxon rank tests will be used to determine the significant difference on baseline SHP-2 overexpression between responders and non-responders.

8.5 Evaluation of Safety

Analyses will be performed for all patients having received at least 14 days' worth of study drug. The study will use the NCI CTCAE v4.0.

9 Study Management

9.1 Pre-study Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Before initiating this trial, the Investigator will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, participant recruitment materials, and any other written information to be provided to participants before any protocol related procedures are performed on any participants.

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

9.2 Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g., advertisements used to recruit participants) will be reviewed and approved by the UCSF IRB. Prior to obtaining IRB approval, the protocol must be approved by the Helen Diller Family Comprehensive Cancer Center Site Committee and by the Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

9.3 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants must sign the IRB-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

9.4 Changes in the Protocol

Once the protocol has been approved by the UCSF IRB, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the Investigator and approved by PRC and the IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to IRB approval. In this circumstance, however, the Investigator must then notify the IRB in writing within five (5) working days after implementation.

The Study Chair and the UCSF study team will be responsible for updating any participating sites.

9.5 Handling and Documentation of Clinical Supplies

The UCSF Principal Investigator and each participating site will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The Principal Investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the Principal Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

9.6 Case Report Forms (CRFs)

The Principal Investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety

monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UCSF personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the CRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the Protocol Project Manager.

9.7 Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center DSMC will be the monitoring entity for this study. The UCSF DSMC will monitor the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious". The DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable. See Appendix 2 Multicenter Institutional Studies Data and Safety Monitoring Plan for a Multicenter Study (Phase 2 or 3 Study), for additional information.

9.8 Record Keeping and Record Retention

The Principal Investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by participants, as well as written records of the disposition of the drug when the study ends.

The Principal Investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data (e.g., signed and dated consent forms and medical records, such as progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, CHR correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

9.9 Coordinating Center Documentation of Distribution

It is the responsibility of the Study Chair to maintain adequate files documenting the distribution of study documents as well as their receipt (when possible). The HDFCCC recommends that the Study Chair maintain a correspondence file and log for each segment of distribution (e.g., FDA, drug manufacturer, participating sites, etc.).

Correspondence file: should contain copies (paper or electronic) of all protocol versions, cover letters, amendment outlines (summary of changes), etc., along with distribution documentation and (when available) documentation of receipt.

Correspondence log: should be a brief list of all documents distributed including the date sent, recipient(s), and (if available) a tracking number and date received.

At a minimum, the Study Chair must keep documentation of when and to whom the protocol, its updates and safety information are distributed.

10 Protection of Human Subjects

10.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all participants involved in human experimentation. This is accomplished through the CHR mechanism and the process of informed consent. The CHR reviews all proposed studies involving human experimentation and ensures that the participant's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The CHR also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

10.2 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

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Appendix 1 Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease
		90	Able to carry on normal activity; minor signs or symptoms of disease
1	Symptoms, but ambulatory Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work)	80	Normal activity with effort; some signs or symptoms of disease
		70	Cares for self, unable to carry on normal activity or to do active work
2	In bed < 50% of the time Ambulatory and capable of all self-care, but unable to carry out any work activities Up and about more than 50% of waking hours	60	Requires occasional assistance, but is able to care for most of his/her needs
		50	Requires considerable assistance and frequent medical care
3	In bed > 50% of the time Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated Death not imminent
4	100% bedridden Completely disabled Cannot carry on any self-care Totally confined to bed or chair	20	Very sick, hospitalization indicated Death not imminent
		10	Moribund, fatal processes progressing rapidly
5	Dead	0	Dead

Appendix 2 Multicenter Institutional Studies Data and Safety Monitoring Plan for a Multicenter Study (Phase II or III study)

1. Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all HDF CCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of patient data.
- Review of serious adverse events.
- Monitoring every six months (depending on patient accrual).
- Minimum of a yearly regulatory audit.

2. Monitoring and Reporting Guidelines

All institutional Phase II or III therapeutic studies are designated with a moderate risk assessment. The data is monitored by a DSMC Monitor twice per year with twenty percent of the patients monitored (or at least three patients if the calculated value is less than three).

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The UCSF Coordinating Center will also coordinate monthly conference calls with the participating sites to communicate the review of adverse events, safety data, and other study matters.

The Principal Investigator at the UCSF Coordinating Center will hold the role of Study Chair. The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating sites. The Study Chair will conduct continuous review of data and patient safety and discuss each patient's treatment at monthly UCSF Site Committee meetings. The discussions are documented in the UCSF Site Committee meeting minutes.

Multicenter communication

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The UCSF Coordinating Center will also coordinate monthly conference calls with the participating sites. The following issues will be discussed as appropriate:

- Enrollment information.
- Adverse Events (i.e., new adverse events and updates on unresolved adverse events and new safety information).
- Protocol Violations.
- Other issues affecting the conduct of the study.

Adverse events reporting to the DSMC will include reports from both the UCSF Coordinating Center, as well as the participating sites. The DSMC will be responsible for monitoring all data entered in OnCore® and eFlorence (a FDA 21 CFR part 11-compliant electronic data repository) at the UCSF Coordinating Center and the participating sites as per the study-specific guidelines. The data (i.e., copies of source documents) from the participating sites will

be downloaded into the PC console of OnCore or from eFlorence prior to the monitoring visits in order for the DSMC to monitor the participating site's compliance with the protocol and FDA regulations.

3. Review and Oversight Requirements

3.1 Adverse Event Monitoring

All Grade 3-5 Adverse Events (AEs), regardless of being unexpected or considered to be associated with the use of the study drug will be entered into OnCore®, UCSF's Clinical Trial Management System.

Adverse Events are graded according to the Common Terminology Criteria for Adverse Events (CTCAE) as developed and revised by the Common Therapy Evaluation Program (CTEP) of the National Cancer Institute. Adverse Events are further given an assignment of attribution or relationship to treatment or medical procedure. Attribution categories are:

- **Definite** – The adverse event is clearly related to the investigational agent(s) or medical procedure.
- **Probable** – The adverse event is likely related to the investigational agent(s) or medical procedure.
- **Possible** – The adverse event may be related to the investigational agent(s) or medical procedure.
- **Unlikely** – The adverse event is doubtfully related to the investigational agent(s) or medical procedure.
- **Unrelated** – the adverse event is clearly not related to the investigational agent(s) or medical procedure.

All Grade 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the UCSF Site Committee meetings. All adverse events entered into OnCore® will be reviewed on a monthly basis at the UCSF Coordinating Center Site Committee meetings. All clinically significant adverse events must be reported to the UCSF Coordinating Center by the participating sites within 10 business days of becoming aware of the event or during the next scheduled monthly conference call, whichever is sooner. The UCSF Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s) from the UCSF Coordinating Center and the participating sites.

3.2 Serious Adverse Event Reporting

By definition, an Adverse Event is defined as a Serious Adverse Event (SAE) according to the following criteria:

- Death.
- Life-threatening (i.e. results in an immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.

- Permanent or significant disability/incapacity.
- Gives rise to a congenital anomaly/birth defect, or cancer, or any experience that suggests a significant hazard, contraindication, side effect, or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above.
- Event occurring in a gene therapy study.
- Event that changes the risk/benefit ratio of a study.
- Any other event the Principal Investigator judges to be serious or which would suggest a significant hazard, contraindication, side effect, or precaution.

Serious Adverse Event reporting will be in accordance with the UCSF IRB Regulations and Code of Federal Regulation Title 21 Part 312.32. The SAE will be reported on a Med Watch form.

UCSF IRB website for guidance in reporting serious adverse events:

https://irb.ucsf.edu/adverse-event

FDA website for guidance in reporting serious adverse events:

www.fda.gov/Safety/MedWatch/HowToReport/default.htm

Med Watch forms and information:

www.fda.gov/medwatch/getforms.htm

All Serious Adverse Events are entered into OnCore®, as well as submitted to the IRB (per IRB guidelines) via iRIS®. All SAEs, whether expected or unexpected, must be reported to the UCSF Coordinating Center within 1 business days of becoming aware of the event. The SAEs are reviewed and monitored by the UCSF Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks. The date the SAE was sent to all required reporting agencies will be documented in OnCore®.

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and is determined to be possibly, probably, or definitely related either to the investigational drug or any research related procedure, the Study Chair at the UCSF Coordinating Center or the assigned designee must be notified within 1 business day from the participating site(s) and the Study Chair must then notify the DSMC Chair or qualified alternate within 1 business day of this notification. The reporting procedure is by communication via phone or in person with written documentation of the one-on-one communication via e-mail, with a copy of the e-mail to the DSMC Director.

3.3 Review of Adverse Event Rates

If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert) is noted in the study, the Study Chair at the UCSF Coordinating Center is responsible for notifying the DSMC at the time the increased rate is identified. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator Brochure or package insert.

If at any time the Study Chair stops enrollment or stops the study due to safety issues, the DSMC Chair and DSMC Director must be notified within 1 business day and the IRB must be notified within 10 business days via an iRIS Reporting Form.

Data and Safety Monitoring Committee Contacts:

Thierry Jahan, MD

[redacted]

UCSF HDFCCC

San Francisco, CA 94158

DSMC Monitors

[redacted]

UCSF HDFCCC

San Francisco, CA 94143

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Appendix 3 Prohibited Medications

Drug	Trade name (if applicable)
Boceprivir:	Victrelis
Clarithrymycin	Biaxin
Conivaptam	Vaprisol
Indinavir	Crixivan
Itraconazole	Sporanox
Ketoconazole	Nizoral
Lopinavir	Kaletra
Mibefradil	Posicor
Nefazodone	Serzone
Nelfinavir	Viracept
Posaconazole	Noxafil
Ritonavir	Norvir
Saquinavir	Invirase
Telaprevir	Incivek, Incivo
Telithromycin	Ketek
Voriconazole	Vfend

Appendix 4 Study Drug Diary

Study Drug Diary

Protocol #______________________Name ____________________________________

Please check each day, AM and PM that you take your study medication. If you do not take your medication on the day it is scheduled, please write in the reason. Also write in any side effects that you experience. *It is very important that you keep this calendar and bring it with you when you see your study doctor or nurse.*

Month _________________________________ Year _____________________

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___
AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___
AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___
AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___
AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___	AM ___ PM___

Appendix 5 Specimen Collection and Processing

Specimen Collection

Blood
Peripheral blood obtained by venipuncture will serve as a source of cells for laboratory testing. Blood will be collected at screening and within 5 days prior to planned surgery (up to and including the day of surgery). One (1) purple top EDTA tube will be collected at each time point. Please refer to the laboratory SOPs for processing instructions.

Tissue
Biopsy material from pre-treatment and post-treatment tumor specimens will be taken from either archival tissue or the initial research biopsy and at the time of surgical resection of the primary tumor, respectively. On both occasions, tissue estimated to be equivalent to at least two 4 mm punch biopsies or two 18 to 14 gauge core needle biopsies will be collected. Tumor samples are ideally to be processed within 5-15 minutes of surgical excision. The tumor samples will be separated into two parts and processed as follows: 1) A tissue sample will be placed in a formalin filled plastic container, then embedded into paraffin for further analysis (FFPE). The FFPE sample will be stored in the UCSF Head and Neck Tissue Repository with study identifiers. 2) A fresh tissue specimen will also be banked within this mechanism.

Tissue Processing

FFPE: For head and neck tumors, construction of tissue microarrays (TMAs) and immunohistochemical staining of the patient specimens will be performed at the Pathology Facility of UCSF. These studies will be supervised by our head and neck pathologist, Dr. Annemieke VanZante who directs the Tissue Core of our HNC Specialized Program of Research Excellence (SPORE). Dr. VanZante is experienced in TMA construction and analysis of protein expression. Paraffin embedded tissue blocks will be sectioned at 5μm and stained with H&E for morphologic characterization and to serve as guide slides for TMA construction. For high throughput immunohistochemical analysis and standardization of staining conditions, tissue microarrays (TMAs) will be constructed from formalin fixed paraffin embedded tissue blocks from the tumor biopsies. Using a manual tissue arrayer, (MTA-1, Beecher Instruments, Sun Prairie, WI) 0.6 mm tissue cores will be extracted from each pre and post treatment tumor in triplicate and arrayed on 1-2 recipient paraffin blocks along with normal tonsillar controls. The newly constructed array block will then be warmed to 35-37°C for 10 minutes to allow annealing of donor cores to the paraffin wax of the recipient block and minimize core loss. Donor cores will range from 2-4 mm in length. Hence each TMA will yield an estimated 100 to 200 tissue sections (4μm thickness). For TMA quality assessment morphologic confirmation of tumor, throughout the entire thickness of the block, one hematoxylin and eosin stained slide will be prepared from every ten tissue sections. Immunohistochemical evaluation of the remaining deparaffinized sections will be performed using immunoperoxidase staining for proteins in the JAK/STAT pathway including the following antigens: JAK1, JAK2, STAT1, STAT3, cyclin D1 and Src on paraffin sections cut from the TMAs using commercially available antibodies and standard methods. Sections will be deparaffinized with successive ethanol and xylene baths. These sections will then be subjected to an optimized antigen retrieval method individualized to each antibody. Signal amplification will be performed using a proprietary micropolymer peroxidase (ImmPRESSTM, Vector, Burlingame, CA) conjugated to an anti-mouse antibody. Immunoreactive cells will be visualized with the brown color resulting from incubation with diaminobenzidine (DAB) chromogenic substrate at room temperature for 5 minutes. Sections

will be counterstained blue with hematoxylin for 15 seconds and lithium carbonate for 5 seconds to provide morphologic detail. Immunohistochemical staining will be scored quantitatively for each core, based upon proportion of immunoreactive cells and staining intensity, with the assistance of computerized digital imaging (Aperio). When appropriate, separate scores for each case will be assigned for cytoplasmic and nuclear staining.