Protocol Title: Pharmacokinetic (PK) Analysis of Antitumor B in Patients With Oral Cancer

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PK Analysis of Antitumor B in Patients with Oral Cancer

Short Title:

Stuart J. Wong, MD (Study PI)

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Title: PK Analysis of Antitumor B in Patients with Oral Cancer

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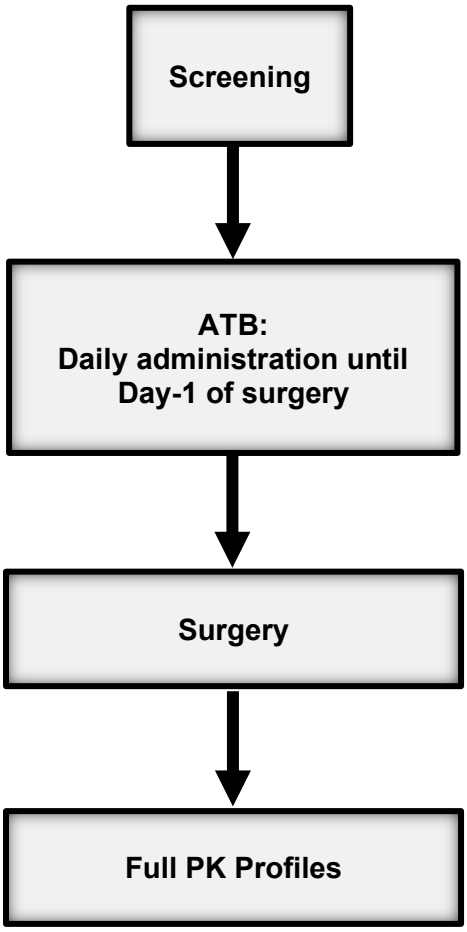
PROTOCOL SUMMARY

Title	PK Analysis of Antitumor B in Patients with Oral Cancer
IND Sponsor	Sponsor Investigator
Funding Source	NCI, R01 CA205633 (M. You, contact PI)
Principal Investigator	Stuart J. Wong, MD
Study Sites	Froedtert & the Medical College of Wisconsin
Clinical Trial Phase	Phase 0
Study Disease	Squamous cell cancer of the oral cavity
Main Eligibility Criteria	▪ Histologically confirmed squamous cell cancer of the oral cavity (including variants of squamous cell histologies). ▪ New diagnosis of oral cancer for which definitive surgery is planned ▪ Prior diagnosis and treatment of head and neck cancer is allowed. ▪ ECOG Performance status < 2. ▪ Age ≥ 18 years. ▪ Normal organ function
Study Rationale	Establishing the PK profile for Antitumor B (ATB) is a critical initial step before future clinical trials can be performed that examine anti-cancer and cancer preventive effects of ATB.
Primary Objectives	To examine the pharmacokinetic properties of ATB.
Study Design	A window of opportunity clinical trial. This study design permits examination of effects of an oral agent on cancer patients during the "window" between diagnosis of their cancer and their definitive cancer surgery. Similar to a phase 0 study, the trial design permits examination of the biologic effects of an agent; in this study pharmacokinetic properties will be examined.
Study Intervention Description	Study participants will take the natural botanical compound ATB during a short window (seven to 28 days). Participants will provide blood samples, and saliva samples during ATB administration and a portion of the initial tumor biopsy.
Number of Subjects	Eight patients will be enrolled to ensure that there will be six analyzable patients.
Estimated Time to Complete Enrollment:	Approximately one year.

STUDY SCHEMA



STUDY CALENDAR

Period/Procedure	Screening	ATB Administration: (Daily till Day -1 of Surgery)			End of ATB administration
Study Day/Visit Day	**-14 to 0 Days[1]**	**Day 1**	**Midway during ATB administration**	**Day -2 to Day -1 of Surgery[13]**	**Day of Surgery**
Informed Consent	X				
Physical Examination[2]	X				X
Medical History[3]	X				
Vitals		X			
Concomitant Medications	X	X		X	X
ATB Administration		X		X	
Pregnancy Test (Serum or Urine)[4]	X				
ECOG Performance Status[5]	X				X
AE Assessment	X	X		X	X
Complete Blood Count (CBC) With Differential and Platelet Count	X				X
PT, INR				X	
Complete Metabolic Panel[6]	X		X[12]	X	X
Research Blood[7]		X			
Research Saliva Samples[8]		See footnote 8 for research saliva sample draw timepoints			
Tumor Specimen[9]	X				X
Patient Diary[10]		X		X	
Compliance Assessment[11]				X	

1. Screening procedures must occur within 14 days prior to registration. ATB administration should start within seven days of registration.
2. Focused physical examination
3. Capture medications taken within 14 days of day 1 of ATB administration
4. For women of childbearing potential.
5. Refer to Appendix 1.
6. Including albumin, total protein, total bilirubin, direct bilirubin, ALT, AST, LDH, alkaline phosphatase, bicarbonate, sodium, potassium, chloride, creatinine, magnesium, calcium, BUN, and glucose.
7. Day 1: Blood samples will be taken at baseline (prior to study agent administration), then at 30, 60, 120, 180, 240, 360, 480, and 1,440 min (a total of nine points). For each blood draw 1 ml of blood will be drawn to yield approximately 500 µl or 0.5 ml of plasma. All blood sample collections are allowed a window of ± 10 minutes. **There will only be ONE ATB dose on day 1. Dose on day 2 must be delayed until completion of PK specimen (blood and saliva) collection**.
8. Day 1: Saliva samples (1 ml) should be collected at baseline (prior to study agent administration), then at 30, 60, 120, 180, 240, 360, 480, and 1,440 min (a total of nine points). Note: 30 min time point has ± 5 min window, all other time points have ± 10 min window. There will only be ONE ATB dose on day 1. All saliva sample collections are allowed a window of ± 10 minutes. Day 2 to day -1 of surgery: A total of at least three patient samples (saliva) will be collected for each time point (predose, before lunch, and before dinner).
9. Refer to Section 5.1.
10. Patient will complete study diary (Appendix 2) with all relevant information and will submit the diary to study coordinator.
11. Study coordinator will contact patients twice weekly for compliance assessment and reminders for protocol procedures.
12. Chemistry panel with AST, ALT, T. Bilirubin will be performed approximately midway during treatment.
13. The indicated assessment for this timepoint will be collected only once with a window of 2 days: Day -2 to Day -1 of surgery.

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LIST OF ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
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
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTO	clinical trials office
DFS	disease-free survival
DLT	dose-limiting toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	data and safety monitoring plan
FDA	Food and Drug Administration
GCP	good clinical practice
HCT	hematocrit
HNC	head and neck cancer
HNSCC	head and neck squamous cell carcinoma
HPV	human papilloma virus
ICF	informed consent form
IRB	Institutional Review Board
LDH	lactate dehydrogenase
MCWCC	Medical College of Wisconsin Cancer Center
NCI	National Cancer Institute
ORR	overall response rate
PBMC	peripheral blood mononuclear cells
PD	disease progression
PK	pharmacokinetics

PR	partial response
RBC	red blood cell (count)
SAE	serious adverse event
SD	stable disease
SD	standard deviation
SOP	standard operating procedure
SRC	Scientific Review Committee
ULN	upper limit of normal
UP	unanticipated problem
UPIRSO	unanticipated problems involving risks to subjects or others
WBC	white blood cell (count)
ATB	antitumor B
SCC	squamous cell carcinoma
TLC	thin layer chromatography
WOO	window of opportunity
CrCl	creatinine clearance
COPD	chronic obstructive pulmonary disease
IND	Investigational New Drug
ICH	International Conference on Harmonisation

1 BACKGROUND

1.1 Antitumor B (ATB)

One of the most promising chemopreventive agents to prevent head and neck cancer is Antitumor B (ATB), a Chinese herbal mixture. It is a botanical agent composed of six Chinese herbs: *Sophora tonkinensis, Polygonum bistorta*, *Prunella vulgaris, Sonchus brachyotus, Dictamnus dasycarpus*, and *Dioscorea bulbifera.* ATB is formulated as 300 mg tablets (Table 1), (8) and manufactured in a GMP facility by National Engineering and Research Center for TCM Shanghai Traditional Chinese Medicine Technology Co. Ltd. (Shanghai, China). Previously, Dr. You and Dr. Hu were able to secure a 20-kg batch of ATB for their proposed study in a funded NIH grant (NIH AT003203), and as such we are confident that we will again be able to secure a large single batch of ATB, enough for its exclusive use in this grant. ATB appears to be very effective in the chemoprevention of upper aerodigestive tract tumors in humans and also safe to several thousand subjects over a period of more than two decades (9–11). Earlier clinical studies conducted in China showed that use of ATB reduced the cancerization rate of marked esophageal dysplasia by 50%. (9–11) In this remarkable clinical trial, more than 2,500 cases of marked esophageal dysplasia were randomly divided into groups for ATB and placebo, respectively. (9–11) After three or five years of treatment, the progression of esophageal dysplasia to esophageal cancer was inhibited by 52.2% and 47.3%, respectively. (9–11)

Table 1. Botanical Raw Materials of Antitumor B (mg per 300 mg extract)

Herbal name		**Plant parts used**	**Form**	**Content (mg/300 mg)**	
Latin name	**Chinese name**			**Range**	**Midpoint**
Sophora tonkinensis Gagnep.	Shan Dou Gen	Dried roots and taproot	Water extract	54-72	63
Polygonum bistorta L.	Quan Shen	Dried rhizome	Water extract	51-63	57
Sonchus brachyotus DC.	Bei Bai Jiang	Dried whole plant	Water extract	51-69	60
Prunella vulgaris L.	Xia Ku Cao	Dried flower stem	Water extract	54-75	64.5
Dioscorea bulbifera L.	Huang Yao Zi	Dried rhizome	Water extract	9-18	13.5
Dictamnus dasycarpus Turcz.	Bai Xian Pi	Dried root bark	Powder	24-36	30
Total				243-333	288

A randomized study of ATB as a preventive agent in patients with oral leukoplakia. Since oral SCC has etiology, histopathology, and molecular mechanism similar to esophageal SCC, it is likely that ATB may be effective for oral cancer chemoprevention. A recent study by Sun Z et al. examined the chemopreventive effects of ATB in a short-term clinical trial of oral leukoplakia. (8). In a three-year period, they recruited 120 patients with oral leukoplakia in Beijing, China and

administered them either ATB tablets or placebo tablets orally. The treatment regimen was 4 (300 mg) tablets each time, three times per day for eight to 12 months. In the entire trial, they conducted clinical evaluations at the study entry time, monthly during the intervention, and three months after the study period; they recorded oral leukoplakia lesions with color photos and measured sizes. They defined a positive response as a disappearance or reduction of size by more than 50% at the final checkup, stable disease as an insignificant change in the size, and progressive disease as an increase in the size of the lesion by >50% or the development of new lesions. In the end, they found that the ATB intervention reduced the size of oral leukoplakia in 40 out of 59 patients whereas the placebo was effective in 9 out of 53 patients.(8) The difference was significant ($p < 0.01$). Such an effect was associated with a significant decrease in the labeling indexes of biomarkers of cell proliferation (AgNOR and PCNA).(8)

ATB Dose and Schedule

ATB has been administered in clinical studies for upper aerodigestive disease prevention in wide ranges of agent dose and schedule, summarized in Table 2 below. Tablet strength in all of these studies was 300 mg. The dose range used in clinical trials was from 2400 mg/d to 4800 mg/d. The duration of therapy ranged from two months to five years.

Study description	Dose/schedule
SATCM (State Administration of Traditional Chinese Medicine) Multicenter Trial, 380 hospitals, 24,275 pts	8 tabs BID (4,800 mg/d) x2 months ON, 1 week OFF= one cycle Duration 2 years
Oral Leukoplakia n=150 ATB; control =148	8 tabs BID (4,800 mg/d) x 2 months
Oral Leukoplakia. n=59 ATB; n=53 placebo (8)	4 tabs TID (3,600 mg/d)
Henan province 1983 Esoph dysplasia, ZSP N=744 agent; n=777 placebo	8 tabs QD (2,400 mg/d) x 5 years
1984 Hebei Province N=300 randomized to ZSP and n=149 control	8 tabs BID (4,800/d) x 6 months

Table 2 Summary of clinical trials illustrating dose and schedule of ATB.

In the current trial, the dose of ATB is in the mid-range (3,600 mg/d), but significantly shorter duration, compared to other ATB studies.

Toxicity

Toxicology studies performed in China, translated and summarized in the enclosed ATB Health Canada Application Supporting Documentation, indicates very low acute toxicity and no obvious pathologic changes in organs from chronic toxicology testing, as well as no mutagenic effects from the Ames test.

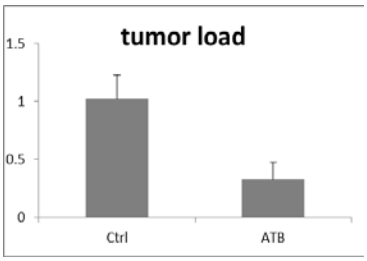
Previous studies of ATB, described in the ATB Health Canada Application Supporting Documentation, have been performed in China for prevention of upper aerodigestive cancer prevention. These studies were placebo controlled and utilized long duration of therapy from two months to five years. In China, there is a general acceptance and common use of natural compounds to treat and prevent disease. The style of reporting of clinical trials of natural compounds reflects this difference compared to the West. Toxicity is qualitatively described in these trials as having low rates of adverse events and high compliance rates even for duration of administration lasting years. In the Hebei Province study of 300 participants randomized to ATB (ZSP) for a duration of six months eight tablets BID (4,800 mg/d), no toxicity was observed including no effects in blood, liver, or kidney function. In the largest trial (24,275 patients), the SATCM study, blood parameters were reported on a subgroup of 349 patients from the SATCM study, who received ATB for three months following completion of definitive chemoradiation for cancer treatment, Minor or non-significant changes were observed in blood parameters. The

Item	Before Treatment	After 3 month	P
White Blood Cells (10^9)	6.14+2.22	5.62+1.59	<0.05
Red Blood Cells (10^{12})	4.73+0.84	4.57+0.43	>0.05
Hemoglobin (g/l)	121.12 +21.88	121.74+16.63	>0.05
Platelets (10^9)	170.65+8.33	159.43+7.09	<0.05

Table 3. Effect of ATB (ACAPHA) on blood parameters following chemoradiation

authors of the SATCM study concluded that overall ATB had little associated little acute of chronic toxicity (“some” patients with loose stool, and a “few” with jaundice and rash). They conclude further that ATB was safe for long-term administration. The SATCM study included patient-reported questionnaires that surveyed a large array of upper intestinal symptoms associated with esophagitis and GERD pre- and during therapy. The study showed improvement of symptoms in the ATB group compared to control. Although previous clinical trials did not specifically examine the effects of ATB on surgical risk, past studies had included biopsies without report of bleeding or healing complications. As a safeguard, however, we have included in our amended protocol, a pre-operative coagulation test.

Preclinical Efficacy



Chemopreventive Efficacy of ATB in 4NQO-Induced Oral Carcinogenesis. We evaluated the chemopreventive efficacy of ATB on the development of 4-nitroquinoline-1-oxide (4NQO)-induced oral SCC in A/J mice.(12) A progression protocol was used in this study by which administration of ATB was initiated in the diet (at 3:7 ratio in AIN76A powder diet) for eight weeks following the first dose of 4NQO. The ATB-containing food was the sole food supply for mice eight weeks after the first dose of 4NQO and continued for 24 consecutive weeks. The details of the experiment are described in our recent paper.(12) In the paper, we showed that ATB inhibited 4NQO-induced oral cancer development by ~60%. In addition, we did not observe any body weight changes in ATB-treated mice. To investigate the effects of ATB on cell proliferation, we quantified Ki-67 stained cells in normal, dysplastic, and SCC oral epithelium and found that ATB inhibited Ki-67 in both dysplasias and SCCs.(12) Recently, we have shown ATB exhibited a significant inhibitory effect on benz(a)pyrene [B(a)P]-induced lung tumorigenesis in A/J mice using the human equivalent dose of ATB (4 g/kg which equals to 12 mg/mouse by oral gavage) in the diet ($p < 0.05$; **Fig 1**), which use a lower dose than our published study but was equally as effective.

Fig 1. Inhibition of B(a)P-induced lung tumorigenesis in A/J mice using human equivalent dose of ATB (4g/kg) in the diet.

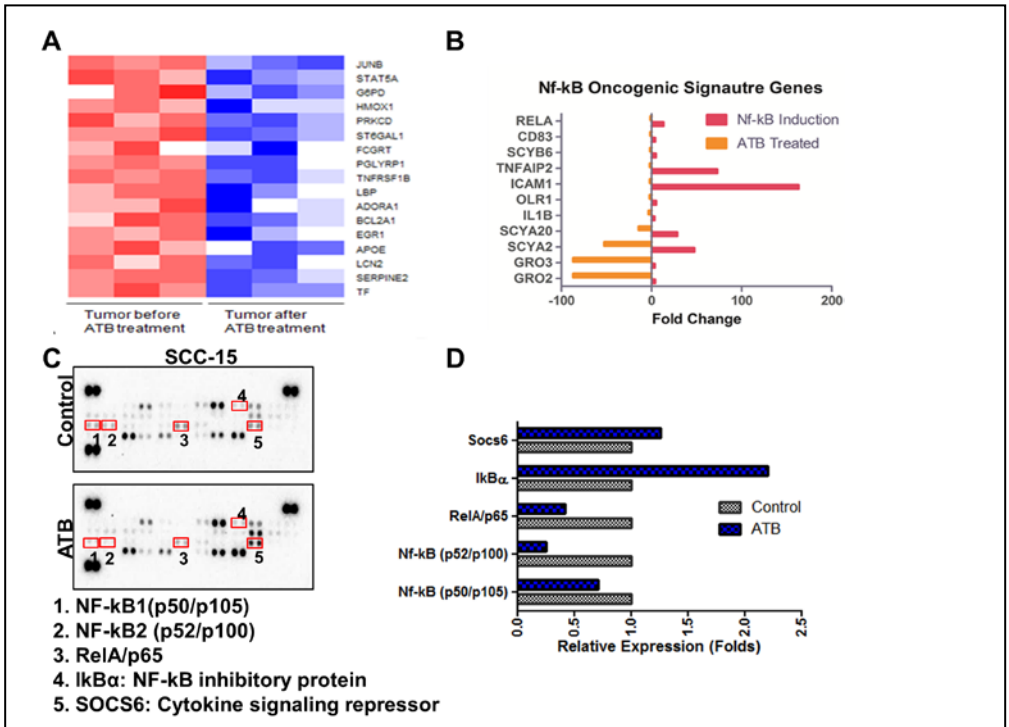


Figure 2

A) Heatmap showing the suppression of NF-κB direct binding biomarkers by ATB in mouse lung tumors; color scheme: red – high expression, blue – low expression, white – middle-level expression; B) 11 NF-κB induced human oncogenic signature genes (from MSigDB) were significantly inhibited by ATB in mouse lung tumors, indicating the suppression of NF-κB signaling; C) NF-Kb proteomic array indicated that ATB inhibited NF-κB signaling activating proteins (both p50/105,p52/p100 and RelA/p65) while upregulated NF-κB signaling inhibitory proteins (IkBα and SOCS6). D) Quantification of NF-kB proteomic array results in C. Notably, ATB treatment in SCC15 cells induced IkBα expression levels more than two folds while inhibited p50/105, p52/p100 and RelA/p65 expression levels compared to that of vehicle control treated SCC15 cells.

ATB Mechanism of Action

ATB Inhibits Oral Cancer Cell Tumorigenesis via Inhibition of NF-kB Signaling Pathway. As the NF-κB signaling pathway is known to play a major role in oral cancer tumorigenesis, (13–16) we examined the effects of ATB in NF-κB signaling pathway via NF-κB proteomic array in

order to validate the RNA seq result (Fig 2 C, D). As predicted from the RNA seq, ATB inhibited NF-κB signaling pathway as NF-κB inhibitory proteins IkBα and SOCS6 were upregulated while NF-κB activating proteins (both p50/p105 and p52/p10) and RelA were downregulated in ATB treated SCC15 oral cancer cells compared to that of vehicle control treated cells (Fig. 2). Growth of oral cancer cells, SCC9 and SCC15 was also inhibited to ATB treatment in a dose-dependent manner (Fig. 3A). We also further validated the effects of ATB on NF-κB signaling in SCC9 oral cancer cells via Western blot (Fig. 3C), which confirmed that ATB treatment downregulated key components of NF-κB signaling pathways in SCC9 oral cancer cells. The functional role of the NF-κB signaling pathway in ATB efficacy was further validated with H1299 lung cancer cells expressing dominant negative IkBα. Growth rate was significantly inhibited in H1299 cells expressing IkBα dominant negative compared to that of control and they were resistant to ATB treatment whereas control H1299 cells were sensitive to ATB treatment in terms of cell proliferation (Fig. 3B). It has been reported that expression of dominant negative IkBα suppressed oral cancer proliferation, survival and tumor growth both *in vitro* and *in vivo,* (17) which indicate the important role of NF-kB signaling pathway in oral cancer tumorigenesis. We are performing the same experiments with SCC9 and SCC15 oral cancer cell lines expressing dominant negative IkBα and a similar result is expected. Therefore, we propose that NF-κB pathway plays a key role in mediating the efficacy of ATB in oral cancer.

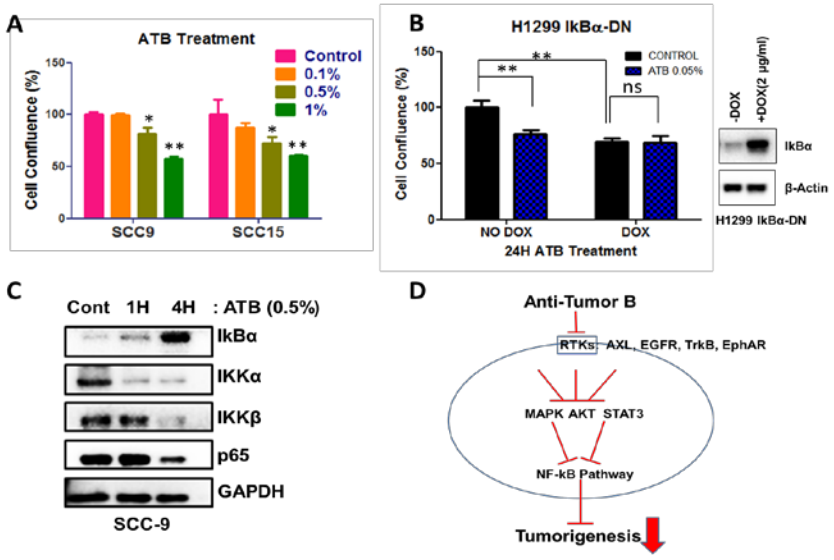


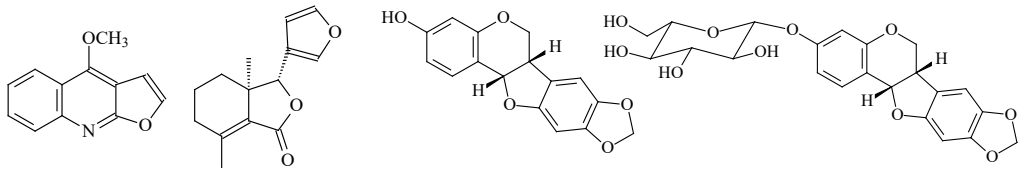
Fig 3. Proposed Mechanism of ATB in the Inhibition of HNSCC Tumorigenesis
A) ATB inhibited cell proliferation of both SCC9 and SCC15 in doses dependent manner. B) Induction of I IkBα dominant negative in H1299 lung cancer cells abrogated anti-proliferative effects of ATB compared to that of control H1299 cells. C) SCC9 oral cancer cells treated with ATB and NF-κB pathway were examined by western blot. ATB inhibited NF-κB pathway in SCC9 oral cancer cells compared to that of cells treated with vehicle control. D) Proposed mechanism of ATB in the inhibition of HNSCC tumorigenesis based on the preliminary data.

Although our preclinical data are compelling regarding the efficacy of ATB as a cancer preventive agent, it is necessary for us to first examine pharmacokinetic properties of ATB to **test the hypothesis that oral administration of ATB will have the desirable PK properties in humans.**

1.2 ATB Components

There are six plants in ATB, described above, and the presence of each of the plants can be verified using a standard thin layer chromatography (TLC) method based on official *Compendium of Chinese Pharmacopeia*. However, the only compound used in the product's current quality control criteria was matrine, which was only moderately active against lung cancer cell line LM1. To identify additional compounds useful for QA and QC purposes, we performed activity guided fractionation of ATB,(18) and identified the following three compounds from a more active fraction GS409 using oral cancer cell line SCC2095: fraxinellone, dictamnine and maackiain (see Fig. 4

for structures). Trifolirhizin is also expected to be highly active because it is rapidly bio transformed to maackiain *in vivo.* (19)



Dictamnine Fraxinellone Maackiain Trifolirhizin

Fig 4. Chemical structures of key active compounds present in ATB and active against lung tumor cell lines LM1 and/or oral cancer cell line SCC2095.

1.3 Window of Opportunity Study Design

Window of opportunity (WOO) trial design,(23) similar in concept as a phase 0 trial design, is well established and is typically used in the early development of novel targeted anti-cancer agents. (23) Employing this trial design, a study participant will receive administration of a natural compound for a short duration during the standard waiting period prior to their definitive anti-cancer treatment, which is nearly always surgery in the case of oral cancer. Traditional clinical endpoints (such as objective response) are typically not the primary objective in WOO or phase 0 trials.

Most commonly, the primary objective is to determine whether an agent achieves its desired or predicted PD/PK parameters (as proposed here). The utility of these biomarker-based early phase trials for oral premalignancy, such as proposed here, has been highlighted by Szabo. (24)

The clinical trial study design, proposed here, is shown in study schema. This study will enroll patients with resectable stage I-IVB squamous cell cancer of the oral cavity who are candidates for surgical tumor resection. Participants will agree to take the study agent for seven to 28 days, allowing collection of specimens for pharmacokinetic testing (blood, saliva, and tumor tissue).

1.4 Head and Neck Cancer

Head and neck cancer refer to a group of biologically similar cancers originating from the upper aerodigestive tract, including the lips, oral cavity, nasal cavity, paranasal sinuses, pharynx, and larynx. Most head and neck cancers are SCC originating from the flattened epithelium of these regions. Head and neck cancer accounts for about 3–5% of all malignancies in Western countries with cancer of the oral cavity accounting for 30% of all head and neck cancers. Oral cancer is the sixth most frequent cancer in the world and approximately 36,500 new cases are diagnosed and 8,000 patients die annually in the United States from this disease. (1) Squamous cell carcinomas (SCC) are the most common malignant neoplasm of the oral cavity. Surgical resection is the primary treatment for SCC of the oral cavity. More than 90% of oral cancer is SCC, of which most have premalignant epithelial lesion stages, such as oral leukoplakia and erythroplakia. (2, 3) The survival rate of patients with HPV negative and oral cavity SCC of the head and neck (HNSCC) remains poor. (4, 5) Despite significant advancements in chemotherapy and radiation therapy, five-year survival rates for these patients have not changed markedly in the past two decades (6,

7). Development of effective chemopreventive interventions of HNSCC is, therefore, urgently needed. ATB may have efficacy in treating or preventing HNSCC, however establishing pharmacokinetic properties is an essential first step.

2 HYPOTHESIS AND OBJECTIVES

We hypothesize that oral administration of ATB will have the desirable PK properties in humans. We propose to examine pharmacokinetic properties of ATB.

2.1 Primary Objectives

To examine pharmacokinetic properties of ATB for short course duration of seven to 28 days.

3 STUDY DESIGN

3.1 General Description

A window of opportunity (WOO) clinical trial permits examination of effects of study agent on cancer patients during the "window" between diagnosis of their cancer and their definitive cancer surgery. Similar to the phase 0 study design, the WOO trial design permits examination of the biologic effects of a study agent; in this trial we will examine PK properties of ATB.

Blood samples will be taken at the following time points Day 1: Blood samples will be taken at baseline (prior to study agent administration), then at 30, 60, 120, 180, 240, 360, 480, and 1,440 min (a total of nine points). For each blood draw 1 ml of blood will be drawn to yield approximately 500 µl or 0.5 ml of plasma. All blood sample collections are allowed a window of ± 10 minutes. A blood sample for liver function test will be performed following completion of ATB on day -1 or day of surgery.

Saliva samples (2-4 ml) will be collected at the following time points: Day 1: Predose, then at 30, 60, 120, 180, 240, 360, 480, and 1440 min (a total of 9 points). A ± 5 minute window is allowed for the 30 min time point. All other time points will have a ± 10 min window. However every effort should be made to obtain samples at the precise time. Day 2 to day -1 of surgery: A total of at least three patient samples (saliva) will be collected for each time point (predose, before lunch, or before dinner). Participants have no limitation on eating or drinking prior to saliva sample collection. Saliva samples will be collected in a 5 ml bio vial and then frozen and stored in an airtight container at 0 to -20^{o} C until batch shipment to Dr. Hu's laboratory at the University of Houston. The blood, saliva, and tumor tissue specimen will be used for PK analysis and should be shipped in dry ice to the following location:

Ming Hu, PhD
Department of Pharmacological and Pharmaceutical Sciences
College of Pharmacy
1441 Moursund St.
University of Houston
Houston, Texas 77030
Contact Information: 832-842-8320

Participants will continue ATB administration for a total of seven to 28 days, which they will record in a study diary (Appendix 2). ATB administration must not exceed more than 28 days. The study diary will be collected at the completion of the study.

At the end of the course, patients will be asked to return the storage specimens to the study coordinator. Patients will be given a Styrofoam transportation container with freeze packs for this purpose. All the specimens will be collected on the day of their surgery.

3.2 Number of Patients

The study team will enroll eight patients in the study.

3.3 Study Duration

Based upon the duration of funding and a conservative estimate of accrual the duration of the clinical trial will be approximately one year.

3.4 Study Completion

The study will reach completion after the last evaluable patient completes protocol therapy.

4 SUBJECT PARTICIPATION, DISCONTINUATION AND WITHDRAWAL

MCW personnel must follow all MCW IRB requirements and policies regarding subject participation, found here:

https://www.mcw.edu/HRPP/Policies-Procedures.htm

4.1 Subject Status

Subject statuses throughout the trial are defined as follows:

- Prescreening: pre-consent (subject considering trial or study staff considering patient for the trial per institutional recruitment methods).
- Screening: period after consent, but prior to eligibility confirmation.
- Consented: consented, prior to eligibility confirmation.
- Eligible: the local investigator confirms all eligibly criteria apply.
- On study/enrolled: date eligibility is confirmed.
- On arm: date of enrollment.
- On treatment: first day treatment was given to the last day treatment was given.
- Off treatment: the last day treatment was given.
- On follow up: from last day of treatment to the end of follow-up period.
- Off study: follow-up period completed, with no additional data gathered.
- Withdrawn: subject fully withdraws consent (i.e., refuses ALL follow-up, even survival) or is taken off study by the local PI.

4.2 Prescreening and Screening Log

The MCW study PI regularly reviews screen failure reasons to understand barriers to accrual and consider amending eligibility criteria. Screen failures are defined as participants who were considered for the trial to participate in the clinical trial with or without consent but are not subsequently assigned to the study intervention or enrolled in the study. MCWCC CTO will follow its SOPs regarding prescreening and screening tracking.

4.3 Consent

Investigators or their appropriate designees will identify potentially eligible subjects from their clinics, subject self-referrals, referrals from other clinicians and/or other IRB-approved recruitment methods. No study conduct, including subject prescreening, can occur until after IRB approval.

A written, signed informed consent form (ICF) must be obtained before any study-specific assessments are initiated. A signed ICF copy will be given to the subject and a copy will be filed in the medical record (per local IRB policies and SOPs). The original will be kept on file with the study records.

After consent, an OnCore® new subject entry occurs must occur within 24 hours of consent.

4.4 Screening Procedures

Refer to the study calendar of events.

Visit procedures that were performed as standard of care prior to consent (without the specific intent to make the subject eligible for the trial), may count toward screening tests and eligibility if they are within the screening window.

4.5 Eligibility Criteria

Study staff must adhere to MCWCC CTO SOPs regarding eligibility review/confirmation.

No waivers of protocol eligibility will be granted, but when clinical factors relating to an eligibility item are unclear or questionable, the study PI (Stuart Wong, swong@mcw.edu) can only provide guidance or clarification on eligibility.

Inclusion Criteria

1. Clinical diagnosis of oral cavity squamous cell cancer.
2. Patient can start study agent administration but histological confirmation of squamous cell cancer (SCC) of the oral cavity (or histologic variants of SCC) by the pathologist must happen within seven days of registration in order to continue study agent administration.
3. Clinical stage II-IVA (as defined by the AJCC, 8th Edition) and amenable to surgical resection
4. New diagnosis of oral SCC, new second primary, or recurrent oral SCC following a minimum remission of six months following previous definite surgery.
5. History and physical examination by an otolaryngologist and medical oncologist within 14 calendar days of study registration.
6. Study agent administration should start within seven days of registration.
7. Patient must receive administration of study agent for a minimum of seven days.
8. ECOG Performance status $\leq$ 2.
9. Age ≥ 18 years.
10. CBC/differential obtained within 14 calendar days prior to registration, with adequate bone marrow function defined as follows:
    Absolute neutrophil count (ANC) ≥ 1,500 cells/mm^3;
    Platelets ≥ 100,000 cells/mm^3;
    Hemoglobin ≥ 8.0 g/dl (Note: The use of transfusion or other intervention to achieve Hgb ≥ 8.0 g/dl is acceptable.);
11. Adequate renal and hepatic function within 14 calendar days prior to registration, defined as follows:
    Serum creatinine < 1.5 mg/dl or creatinine clearance (CrCl) ≥ 50 ml/min within 14 calendar days prior to registration, determined by 24-hour collection or estimated by Cockcroft-Gault formula:
    CrCl male = [(140 – age) x (wt. in kg)], [(Serum Cr mg/dl) x (72)]
    CrCl female = 0.85 x (CrCl male)

CRC Initials: _____ Date: _____

Investigator/Enrolling Physician Initials: _____ Date: _____

12. Total bilirubin < 2 x the institutional ULN within 14 calendar days prior to registration.
13. AST or ALT ≤ 3 x the institutional ULN within 14 calendar days prior to registration.
14. Magnesium, calcium, glucose, potassium, and sodium within 14 calendar days prior to registration, with the following required parameters:
    Magnesium: > 0.9 mg/dl or < 3 mg/dl;
    Calcium: > 7 mg/dl or < 12.5 mg/dl;
    Glucose: > 40 mg/dl or < 250 mg/dl;
    Potassium: > 3 mmol/L or < 6 mmol/L;
    Sodium: > 130 mmol/L or < 155 mmol/L.

15. Female patients must meet one of the following:
    - Postmenopausal for at least one year before the screening visit, or
    - Surgically sterile (i.e., undergone a hysterectomy or bilateral oophorectomy), or
    - If subject is of childbearing potential (defined as not satisfying either of the above two criteria), agree to practice two acceptable methods of contraception (combination methods requires use of two of the following: diaphragm with spermicide, cervical cap with spermicide, contraceptive sponge, male or female condom, hormonal contraceptive) from the time of signing of the informed consent form through 90 days after the last dose of study agent, AND
        - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptom-thermal, post ovulation methods] and withdrawal are not acceptable contraception methods.)

16. Male patients, even if surgically sterilized (i.e., status postvasectomy), must agree to one of the following:
    - Practice effective barrier contraception during the entire study period and through 60 calendar days after the last dose of study agent, OR
    - Must also adhere to the guidelines of any study-specific pregnancy prevention program, if applicable, OR
    - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptom-thermal, post ovulation methods] and withdrawal are not acceptable methods of contraception.)

17. Patients must be deemed able to comply with the study plan.
18. Gastric tube study agent administration is permissible.
19. Patients must provide study-specific informed consent prior to study entry.

CRC Initials: _____ Date: _____

Investigator/Enrolling Physician Initials: _____ Date: _____

Exclusion Criteria

1. History of active liver disease.
2. Pregnant or lactating women are ineligible due to unforeseeable risks to embryo or fetus.
3. Concurrent use of any medicinal botanical, natural, or other herbal compound/s that the study PI believes could potentially have an impact on the results/objectives of this study.
4. Planned subtotal or debulking surgery, as determined by enrolling physician determination, is not permissible.
5. Prior systemic chemotherapy for oral SCC; note that prior chemotherapy for a different cancer is allowable.
6. Prior radiotherapy for oral SCC is permissible if disease free for one year since prior oral cancer treatment and free of significant late radiation effects.
7. Severe active comorbidity, such as uncontrolled cardiac disease, infection, severe COPD.

I have reviewed all inclusion and exclusion criteria and confirm the subject is eligible.

_____ _____

(CRC Signature) (Date)

_____ _____

(Investigator/Enrolling Physician Signature) (Date)

4.6 Discontinuation of Study Treatment, Withdrawal, and Compliance

Discontinuation from the study treatment does not mean discontinuation from the study. Subject will be considered in follow-up, study procedures should still be completed as indicated by the study protocol, and AEs/SAEs will continue to be reported according to this protocol.

In the absence of treatment delays due to adverse events, study treatment/intervention may continue until:

- Disease progression.
- General or specific changes in the subject's condition renders the subject unacceptable for further treatment in the investigator's judgment.
- Inter-current illness that prevents further treatment administration.
- Subject decides to withdraw from the study.
- The subject has significant noncompliance with the protocol (see below).
- Unacceptable adverse event(s) and/or dose level reduction beyond requirements as detailed in this protocol.
- Study stopping rules are met.

Subjects who sign the informed consent form, enroll and receive the study intervention, but subsequently withdraw, or are withdrawn or discontinued from the study will not be replaced.

Consent Withdrawal

A subject may decide to withdraw from the study at any time. MCWCC CTO will follow its IRB of record's SOPs regarding consent withdrawal.

If a subject intends on withdrawing consent, staff should confirm which of the following options the subject chooses and document the discussion:

- Full consent withdrawal, with no study follow-up.
- Selective consent withdrawal from interventional portion of the study but agree to continued follow-up of associated clinical outcome information.

Investigator-Initiated Withdrawal

The investigator will withdraw a subject whenever continued participation is no longer in the subject's best interests. Reasons for withdrawing a subject include, but are not limited to, disease progression, the occurrence of an adverse event or a concurrent illness, a subject's request to end participation, a subject's noncompliance or simply significant uncertainty on the part of the investigator that continued participation is prudent. The reason for study withdrawal and the date the subject was removed from the study must be documented.

4.7 Lost to Follow-Up

The following actions must be taken if a participant fails to return to the clinic for a required study visit and/or is unable to be reached for follow-up:

- The investigator or designee must make every effort to regain contact and/or reschedule a missed visit with the participant.
- A participant is deemed lost to follow-up if his/her status cannot be obtained after *all* of the following occurs at two consecutive scheduled protocol calendar timepoints:

    - Three telephone calls (at least one day apart) from the study team are unanswered,

        AND

    - A letter to the participant's last known mailing address goes unanswered, **AND**
    - These contact attempts must be documented in the participant's medical record or study file.

- Update OnCore® (Follow-up tab and eCRF) when a participant is officially considered lost to follow-up.
- If a subject is considered lost to follow-up, but subsequently contacts the study team, the subject should be considered in follow-up again.

4.8 Accrual Suspension and Closure

The MCW PI facilitates the suspension and closing of accrual in the following manner:

- OnCore® tracks accrual throughout the study.
- If the study must be suspended, OnCore® is updated to a 'suspended' status.
- When the accrual number is reached, OnCore® notifies staff of study closure.

4.9 End of Study Definition

A participant is considered to have completed the study if he or she completed all phases of the study, including the last visit or the last scheduled procedure shown in the calendar of events or has been discontinued.

4.10 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause (as determined by the MCW study PI, DSMC, sponsor, and/or IRB). Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the MCW principal investigator (PI) will promptly inform the MCW Institutional Review Board (IRB), and the sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes.

5 TREATMENT PLAN

5.1 ATB Administration

ATB will be administered on an outpatient basis. ATB will be administered at a dose of 1,200 mg three times per day (roughly spaced every eight hours) as has been studied for ATB in human oral leukoplakia trials and in esophageal cancer prevention trials. ATB may be taken with or without food.

Regimen Description					
Study agent	**Premedication; precautions**	**Dose**	**Route**	**Schedule**	**Duration**
ATB	None	1,200 mg, Three times a day	Oral	Daily	7-28 days[a]
a. Total duration depends upon scheduling of surgery. ATB administration must not exceed 28 days.					

- **There will only be ONE ATB dose on day 1. Dose on Day 2 must be delayed until completion of PK specimen (blood and saliva) collection**.
- Patient must submit completed study dairy to the study coordinator.

5.2 Supply

ATB will be produced by National Traditional Chinese Medicine Pharmaceutical Engineering Technology Research Center and shipped to MCW in the form of a tablet. ATB will be stored at room temperature in a cool place, free from excessive moisture and should be stable for two years. Analysis of pharmacokinetic properties is necessary before future efficacy trials can be initiated. We will examine an oral ATB dose of 1,200 mg, three times per day.

Study agent will be shipped to MCW in one batch. Study agent ordering contact information:

Ming Hu, Ph.D.
Department of Pharmacological and Pharmaceutical Sciences
College of Pharmacy
1441 Moursund St.
University of Houston
Houston, Texas 77030
Contact Information: 832-842-8320

Each patient-specific bottle will be labeled by IDS pharmacy with the following:

- The study number;
- The bottle number (i.e., Bottle 1 of 2 and Bottle 2 of 2);

- The number of tablets;
- The patient ID number (e.g. ATB-YYY, where the study number and sequence number represent the unique patient identifier assigned at registration);
- The patient's initials (i.e., first, middle, last);
- A blank line for the site pharmacist to enter the patient's name;
- Administration instructions (i.e., "Take xx tablets every day for xx days");
- Storage instructions (i.e., "Store at controlled room temperature, xx degrees");
- Emergency contact instructions.

5.3 Storage and stability

The intact bottles should be stored at controlled room temperature (15°C – 30°C). Shelf life surveillance studies of the intact bottle are ongoing. Current data indicate ATB is stable at 90 days without decrement of active components at controlled room temperature (15°C – 30°C).

5.4 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents. It is the responsibility of the investigator to ensure that the agents are only dispensed to study patients.

5.5 Drug Destruction

Opened bottles of ATB must be disposed of by incineration at the site as chemotherapy or biohazardous waste. At the completion of the study, all unused ATB also must be incinerated at the site. It is the responsibility of the investigator to ensure that a current record of agent disposition is maintained at each study site where agents are inventoried and disposed, including dates and quantities.

5.6 Biopsy specimen, Operative Specimen, and Tissue Processing

Tumor specimens will be analyzed for ATB accumulation and stored for tissue banking for future research. Submission of a block is preferred and is highly encouraged. If a block is not available, please provide at least five unstained slides. The block (or unstained slides) should be hand delivered to the CRI Histology Core (address below): The contact person is Christine Duris (phone number 414-955-8624).

Second post-ATB administration tumor biopsy is not needed because the OR-resected tumor will serve this purpose. At the time of surgery, a portion of the resected tumor will be utilized for the study.

The resected tumor will be received and processed by the MCW tissue bank staff. The tissue will be fixed in 10% buffered formalin as soon as resected, grossed into cassettes and processed into paraffin blocks. Once the blocks are in paraffin – the blocks will be delivered to the CRI Histology Core for further testing. The paraffin block of the resected tumor and biopsy should be delivered to the following location:

Children's Research Institute – Histology Core Laboratory
Medical College of Wisconsin

TBRC-CRI Room C4305
8701 Watertown Plank Road
Milwaukee, WI 53226

The biopsy specimen and tumor specimen will be used for PK analysis.

5.7 Dose Modifications

As indicated in the study calendar, a complete metabolic panel (including LFTs) will be performed mid-way during the course of ATB. Adverse events will be graded according to CTCAE v.5.

Adverse Event (CTCAE, v. 5)	Action
Non-hematological, grade 1 or 2	Continue ATB therapy at full dose prescribed. Apply maximum supportive care recommendations. If persistent duration of grade 2 adverse event is affecting quality of life, a one-time decrease of 25%.
Non-hematological, grade 3 or 4	Apply maximum supportive care recommendations. Hold ATB therapy until recovery to grade $\leq$ 1 (up to 7 days). If concomitant elevation of AST/ALT 3 times the upper limit of normal and bilirubin elevation 2 times the upper limit of normal (Hy's Law criteria) the patient must be removed from study treatment, ATB must be permanently discontinued. If recurrence of adverse event after drug hold and/or interruptions is observed and maximum supportive care measures applied, a dose reduction by 25% is recommended. ATB should not be used at doses below 50%
Non-hematological, grade 3 or 4 and adverse events NOT resolved to grade $\leq$ 2 within a maximum of 14 calendar days from last planned administration	Dose reductions by 25% (up to 2 reductions) will be considered after maximum supportive care recommendations are introduced.

Dose Level	Dose
-2	600 mg TID
-1	900 mg TID
0	1200 mg TID

5.8 General Concomitant Medication and Supportive Care Guidelines

All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol and documented on source documents as concomitant medication.

Concurrent or concomitant use of medication is not restricted during protocol therapy. However, patients are not permitted to take any anti-cancer medications or supplements during study duration.

Few side effects of ATB have been reported. ATB has shown to improve many baseline gastrointestinal symptoms particularly those associated with GERD. However, diarrhea has been rarely reported in relation to ATB administration and should be managed using the following guidelines.

- Loperamide (Imodium®)
  All patients should be instructed to begin taking loperamide at the earliest signs of diarrhea and/or abdominal cramping that occur more than eight hours after receiving ATB. Patients will be instructed to begin taking loperamide at the earliest signs of (1) a poorly formed or loose stool, (2) the occurrence of one to two more bowel movements than usual in one day, or (3) unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at the first onset of diarrhea, then 2 mg every two hours around the clock until diarrhea-free for at least 12 hours. Patients may take 4 mg of loperamide every four hours during the night. Additional antidiarrheal measures may be used at the discretion of the treating physician.

5.9 Dietary Restrictions

No food interactions are known to occur with ATB, thus, no dietary restrictions will be placed upon participants

5.10 Monitoring Subject Compliance

ATB will be administered or dispensed only to eligible patients under the supervision of the investigator or trained study staff or identified sub investigator(s). The appropriate study personnel will maintain records of study agent receipt and dispensing. Comprehensive instructions will be provided to the patient to ensure compliance with dosing procedures.

6 ADVERSE EVENTS: DEFINITIONS, COLLECTION, REPORTING REQUIREMENTS AND SAFETY ASSESSMENT

6.1 Definitions

6.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. (International Conference on Harmonisation [ICH], E2A, E6).

This study will utilize the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, located on the CTEP web site:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

6.1.2 Serious Adverse Event (SAE)

Serious Adverse Event (SAE) means any untoward medical occurrence that results in any of the following outcomes:

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization (see clarification in the paragraph below on planned hospitalizations).
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Pregnancy.**
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. 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; any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

6.1.3 Attribution of an Adverse Event

An assessment of the relationship between the adverse event and the medical intervention, using the following categories:

Definitely Related: *The AE is clearly related to the intervention*. There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

Probably Related: *The AE is likely related to the intervention.* There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

Possibly Related: *The AE may be related to the intervention.* There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events).

Unlikely: *The AE is doubtfully related to the intervention.* A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

Unrelated: *The AE is clearly NOT related to the intervention.* The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology.

6.1.4 Expectedness of an Adverse Event

Study investigator or treating physician will be responsible for determining whether an AE is expected or unexpected as indicated in the protocol, informed consent form and/or drug information brochure. An AE will be considered unexpected if the nature, severity, or frequency of the event is NOT consistent with the risk information previously described for the study intervention.

6.2 Collection and Reporting Requirements for Adverse Events and Serious Adverse Events

6.2.1 Collection of Adverse Events

All (or specify if only certain grade AE needed) adverse events (including SAEs) must be recorded in OnCore® and/or an adverse event log. All AEs required to be collected must be graded according to the CTCAE v5. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. Investigator's or treating physician's assessment of AE attributions must also be documented.

AEs will be collected from the time the subject signs the consent form through 30 days post last dose of study drug(s). AEs will be tracked and followed until resolution, subject withdraws consent, or is lost to follow-up (including subjects who discontinue early). All adverse events collected per the protocol will be followed with appropriate medical management until they are resolved, if they are related to the study treatment, or until the investigator deems the event to be chronic.

Please see section 6.2.2 and table 4 to identify the adverse events that need to be reported.

6.2.2 Reporting of Adverse Events and Serious Adverse Events

Please refer to table 4 below to identify adverse events that meet reporting requirements.

All serious adverse events (SAEs) that occur after the subject has signed the consent form through 30 days post last dose of study drug(s) will be reported. All SAEs will be followed until satisfactory resolution, or until the investigator deems the event to be chronic.

All serious adverse events (SAEs) must also be documented in OnCore®.

Table 4

<table>
<tr><th rowspan="3">Attribution</th><th colspan="4">SAE</th><th colspan="3">AE</th></tr>
<tr><th colspan="2">Grade 1, 2 & 3</th><th colspan="2">Grade 4 and 5</th><th>Grade 3</th><th colspan="2">Grade 4</th></tr>
<tr><th>Expected</th><th>Unexpected</th><th>Expected</th><th>Unexpected</th><th>Unexpected</th><th>Expected</th><th>Unexpected</th></tr>
<tr><td>Unrelated
Unlikely</td><td rowspan="2">IRB[1] and DSMC[2]-Routine Review[3]</td><td>IRB[1] and DSMC[2]-Routine Review[3]</td><td rowspan="2">IRB[1]-Routine Review[3]

DSMC[2]-Within 5 calendar days</td><td>IRB[1]-Routine Review[3]

DSMC[2]-Within 5 calendar days</td><td rowspan="2">DSMC[2]-Routine Review[3]</td><td rowspan="2">DSMC[2]-Within 5 calendar days</td><td rowspan="2">DSMC[2]-Within 5 calendar days</td></tr>
<tr><td>Possible
Probable
Definite</td><td>IRB[1] and DSMC[2]-Within 5 calendar days

FDA[4]</td><td>IRB[1] and DSMC[2]-Within 5 calendar days

FDA[4]</td></tr>
</table>

1. Guidance on adverse event reporting to the IRB is available online at MCW IRB Policies and Procedures.
2. For expedited DSMC reporting, study coordinator/research nurse must notify the DSMC via email including the subject ID, date of event, grade, relatedness, expectedness, and a short narrative. DSMC will review data entered into OnCore®.
3. For routine reporting, the events will be reported to IRB as part of the annual continuing progress report and the DSMC will review data entered into OnCore® at the time of scheduled monitoring.
4. Fatal or life-threatening SAEs meeting the criteria indicated in the above table will be reported to FDA no later than seven calendar days after study staff's initial awareness of the event. If the SAE is not fatal or life-threatening and meets the above criteria, the timeline for submitting an IND safety report to FDA is no later than 15 calendar days after study staff's initial awareness of the event. See section 6.2.3 for detailed reporting instructions.

6.2.3 Reporting Instructions

An IND safety report will be submitted for any adverse event that meets all three definitions: possibly related to the study drug, unexpected, and serious. If the adverse event does not meet one of the above definitions, it should not be submitted as an expedited IND safety report.

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)).

Suggested Reporting Form:

US FDA MedWatch 3500A:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

6.3 Unanticipated Problem Involving Risk to Subject or Other (UPIRSO)

The investigator and his or her team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

6.4 Subject Complaints

If a complaint is received by anyone on the study staff, it will be discussed with the study staff and will be addressed on a case-by-case basis. The PI will be notified of any complaints. Complaints will be reported to the IRB if indicated.

If the subject has questions about his or her rights as a study subject, wants to report any problems or complaints, obtain information about the study or offer input, the subject can call the Medical College of Wisconsin/Froedtert Hospital research subject advocate at 414-955-8844. This information is provided to the subject in their consent.

A product complaint is a verbal, written or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact the drug manufacturer and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a drug manufacturer representative. Product complaints in and of themselves are not reportable events. If a product complaint results in an SAE, an SAE form should be completed.

6.5 Safety Assessment

Dose-limiting toxicity (DLT) is defined as the occurrence of any grade $\geq$ 3 adverse event (AE) that is definitely or probably related to ATB and occurs during the study.

An early safety assessment will be performed after the first four patients complete protocol therapy and reported to the DSMC. In the event of 0-1 DLT events, the experimental therapy is considered safe and the study will proceed to the second cohort of four patients. In the event of $\geq$ 2 DLTs in the first four patients, the study chairs and DSMC will recommend proceeding with the second cohort at dose level -1 or dose level -2.

7 STATISTICAL CONSIDERATIONS

7.1 Statistical Design and Sample Size Calculations

Rationale: Assuming the SD of the PK parameters are approximately 50% of the mean of the PK parameters, which implies an effect size of two, a sample size of six analyzable patients will provide 95% confidence intervals with a margin of error, based on a t distribution with five degrees of freedom, of approximately +/- 52.5% of the mean. These are typical for PK estimation studies.

7.2 Study Objectives and End Points

The endpoint of the study is the successful evaluation of a PK profile, with estimation of PK parameters such as plasma and saliva AUC and Cmax. The study shall be deemed complete when the 6th evaluable patient has completed therapy and the samples sent for PK analysis.

7.3 Statistical Methodology and Analysis Plan

Statistical methodology: PK parameters for drug concentration in both saliva and plasma samples will be calculated. These PK parameters include area under the concentration curves to last collection time (AUCt), maximum measured concentration (Cmax) and time to maximum concentration (Tmax).

8 DATA AND SAFETY MONITORING PLAN (DSMP)

8.1 Data and Safety Management Overview

The Medical College of Wisconsin (MCW) Data Safety Monitoring Committee (DSMC) and the MCW Institutional Review Board (IRB) will approve protocol-specific DSM plans. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with safety and progress reports submitted to the DSMC.

The DSMP for this study will involve the following entities:

8.2 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, regulatory specialist, and the study biostatistician. While subjects are on study, the principal investigator will meet regularly with the research coordinator and the study biostatistician to review study status. This review will include but not be limited to reportable SAEs and UPIRSOs and an update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed. All meetings including attendance are documented.

8.3 Quality Assurance

The MCWCC Clinical Trials Office provides ongoing quality assurance audits. This protocol was classified as high risk and will be reviewed internally by the MCW Cancer Center Clinical Trials Office Quality Assurance Staff according to the MCWCC Data and Safety Monitoring Plan and current version SOP, 6.5.2 Internal Quality Assurance Reviews.8.4 Clinical Trials Office

The MCWCC Clinical Trials Office (CTO) provides administrative assistance and support to the DSMC.

8.5 DSMC

The Medical College of Wisconsin Cancer Center places the highest priority on ensuring the safety of patients participating in clinical trials. Every cancer interventional trial conducted at MCW includes a plan for safety and data monitoring.

More information can be found related to the MCWCC Data and Safety Monitoring Plan at the MCWCC website (Data and Safety Monitoring Plan).

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data and Safety Monitoring Committee (MCWCC DSMC). A summary of the MCWCC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension, or termination as necessary.

Any available DSMC letters will be submitted to the IRB of record as required.

9 REGULATORY COMPLIANCE, ETHICS, AND STUDY MANAGEMENT

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.

9.1 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children), 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 Prestudy Documentation

Prior to implementing this protocol at MCWCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MCW IRB.

The clinical investigation will not begin until the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

9.3 Institutional Review Board

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 MCW Institutional Review Board. Prior to obtaining MCW approval, the protocol must be approved by the Medical College of Wisconsin Cancer Center Scientific Review Committee. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent forms will be IRB-approved and the subject (and Legally Authorized Representative, if necessary) will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. In accordance with 46 CR 46.111, the subject will sign and date the informed consent document prior to any procedures being done specifically for the study.

A witness should only sign when required, per FH/MCW IRB policy. If a witness signs the document when not required, the study staff should document in the legal medical record (or note to file) the relationship to the patient and why a witness signed. (i.e., "Although not required, the subject's spouse was present during the consenting process and signed as the witness." Or "Although not required, hospital staff was present for consenting process and signed as a witness.")

The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial.

A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. If there are changes to the consent form, all revisions will be reviewed with study subject at the next appropriate opportunity. Patients that require reconsenting will be defined in the IRB approved amendment submission. The process for obtaining informed consent will again be performed. Study subjects will not be reconsented for continuing reviews. The MCWCC CTO will follow the MCW/FH IRB's policy for subjects who demonstrate limited English proficiency or limited literacy.

After the subject's visit in which the consent is signed, it is documented in the clinic chart that the consent has been signed and that all questions have been answered to the subject's satisfaction after adequate time for review of the consent. It is also documented that a copy of the consent is given to the subject. The original consent is kept with the subject's study file, and a copy of the consent is sent to the OCRICC office, which will then submit to HIM a copy of the signed consent to be scanned into EPIC, the legal medical record.

9.4 Subject Confidentiality and Access to Source Documents/Data

Subject confidentiality is strictly held in trust by the sponsor-investigator, participating investigators, and any staff, [and the sponsor(s) and their agents] (include bracketed portion if applicable). This confidentiality includes the clinical information relating to participating subjects, as well as any genetic or biological testing.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the principal investigator.

The conditions for maintaining confidentiality of the subjects' records are required for the life of the data. These rules apply equally to any and all MCWCC projects.

One risk of taking part in a research study is that more people will handle the personal health information collected for this study. The study team will make every effort to protect the information and keep it confidential, but it is possible that an unauthorized person might see it. Depending on the kind of information being collected, it might be used in a way that could embarrass the subject or affect his/her ability to get insurance.

While data are being collected and after all data have been collected but are still in the process of being analyzed, the subject's data/PHI are stored in the locked Clinical Research office in the Clinical Trials Office. Databases in which the study subject information is stored and accessed are password protected, allowing for limited access by authorized personnel only. Data/PHI kept in the Case Report Forms contain the study identifiers, subject initials, date of birth and date of service.

The principal investigator will allow access to all source data and documents for the purposes of monitoring, audits, IRB review, and regulatory inspections.

The study monitor/s or other authorized representatives of the principal investigator may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

9.5 Protection of Human Subjects

9.5.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the informed consent process. The IRB reviews all proposed studies involving human experimentation and ensures that the subject'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 IRB 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.

9.5.2 Protection of Privacy

As noted, 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 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.

9.5.3 Changes in the Protocol

Once the protocol has been approved by the MCW 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 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 working days after implementation.

The IRB may provide, if applicable regulatory authority(ies) permit, expedited review, and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB. The investigator will submit all protocol modifications to the sponsor and the regulatory authority(ies) in accordance with the governing regulations.

Changes to the protocol may require approval from the sponsor.

Any departures from the protocol must be fully documented in the source documents.

9.6 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies).

<u>Onsite Audits</u>

Auditing is essential to ensure that research conducted at the Medical College of Wisconsin (MCW) Cancer Center is of the highest quality and meets MCW and regulatory agency standards.

Regulatory authorities, the IRB and/or sponsor may request access to all source documents, data capture records and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

10 DATA HANDLING AND RECORD KEEPING

10.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution, and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss, or theft. This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians, and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

10.2 Data Management Responsibilities

Principal Investigator
The principal investigator oversees the management of patient records/case report forms and ensures that a) complete and accurate data will be obtained and provided to the sponsor; b) patient records are maintained to include history, prescribed medication, and investigational product(s), measurements, exams, evaluations, and adverse events; c) corrections are applied to clinical research data according to principles of good research practice (i.e., single-line delete, date and initial). He or she will ensure that there is correlation between the case report forms and the source documents**.**

Research Coordinator
A research coordinator creates, collects, and organizes clinical trial documentation. He or she ensures that source documentation and data abstraction and entry are being done at protocol specified time points.

Research Nurse/Medical Staff
The research nurse and medical staff documents protocol-required care or assessment of the subject's outcomes, adverse events, and compliance to study procedures.

Biostatistician
The biostatistician may assist in CRF development (content and design), dataset specifications (annotation of CRFs and record layout) and validation.

10.3 Source Documents

Source documents for clinical information (patient history, diagnosis, clinical and diagnostic test reports, etc.) are maintained in the patient's clinical file.

All source documents will be written following ALCOA standards:

ALCOA Attribute	Definition
Attributable	Clear who has documented the data.
Legible	Readable and signatures identifiable.
Contemporaneous	Documented in the correct time frame along with the flow of events. If a clinical observation cannot be entered when made, chronology should be recorded. Acceptable amount of delay should be defined and justified.
Original	Original, if not original should be exact copy; the first record made by the appropriate person. The investigator should have the original source document.
Accurate	Accurate, consistent and real representation of facts.
Enduring	Long-lasting and durable.
Available and accessible	Easily available for review by treating physicians and during audits/inspections. The documents should be retrievable in reasonable time.
Complete	Complete until that point in time.
Consistent	Demonstrate the required attributes consistently.
Credible	Based on real and reliable facts.
Corroborated	Data should be backed up by evidence.

10.4 Case Report Forms

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 outcomes. All study data will be entered into OnCore® via standardized CRFs, in accordance with the study calendar, using single data entry with a secure access account. The clinical research coordinator 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 MCWCC 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 principal investigator will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

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

10.5 Study Record Retention

The principal investigator is required to maintain adequate records.

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.

Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, 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, IRB 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.

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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. STUDY DIARY

Participants will complete study diary to track compliance, saliva specimen collection, and daily symptom documentation. A paper study diary will be provided to each subject at screening to record the following information:

1. Study Agent administration date and time
2. Time of saliva sample collection
3. Side effects and/or any new symptoms

Patients will be trained on use of the diary. The diary will be provided in paper format and remain with the patient for the duration of the study. The study coordinator will review the diary for completeness and request missing information periodically and in a timely manner. Side effects/symptoms recorded in the diary will be reported as AEs according to the investigator's discretion and clinical judgment. The subject diary will serve as a source record and remain at the study site after the subject completes the study.

Study coordinator will contact patients twice weekly for compliance assessment and reminders for protocol procedures. At the end of the course of therapy patients will be asked to return the storage specimens to the study coordinator. Patients will be given a Styrofoam transportation container with freeze packs for this purpose. Patient will complete study diary with all relevant information and will submit the diary to study coordinator on the day of surgery.

STUDY DIARY PATIENT INSTRUCTIONS:

1. Study coordinator will provide you specific instruction on how to complete the diary

2. Record the date, when you took ATB, and when you took them. Record dose as soon as you take them; do not batch entries together at a later time.

3. Record the time of saliva sample collection. Collect saliva sample even if you miss any dose.

4. If you have any comments or notice any side effects, please record them in the Comments/ Symptoms. If you make a mistake while you write, please cross it out with one line, put your initials next to it, and then write the corrected information next to your initials

5. If you miss a dose of ATB, you should take it as soon as possible, as long as it is on the same day. Do not take an extra dose of ATB on the next day or any subsequent days to make it up for the missed dose.

6. Please return this diary to the study coordinator after study completion

Antitumor B (ATB) Medication Diary

Subject # (completed by study staff): _____

Cycle # (completed by study staff): _____

Assigned pill strength # (completed by study staff): _____

Day	Date	Administration of ATB	Administration Time	Time of saliva sample collection (e.g. Pre-dose, before lunch or before dinner)	Comments/ Symptoms
Day1		Dose 1			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			

Antitumor B (ATB) Medication Diary

Subject # (completed by study staff): _____

Cycle # (completed by study staff): _____

Assigned pill strength # (completed by study staff): _____

Day	Date	Administration of ATB	Administration Time	Time of saliva sample collection (e.g. Pre-dose, before lunch or before dinner)	Comments/ Symptoms
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			
		Dose 1			
		Dose 2			
		Dose 3			