ID: UMCC 2010.101	Chemotherapy AND Bcl-xL Inhibitor (AT-101) For Organ Preservation In Adults With Advanced Laryngeal Cancer	NCT01633541

University of Michigan Head and Neck SPORE

CONCOMITANT CHEMOTHERAPY AND Bcl-xL INHIBITOR (AT-101) FOR BIOSELECTION™ FOR ORGAN PRESERVATION IN PATIENTS WITH ADVANCED LARYNGEAL CANCER

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1.0 Introduction:

1.1 Larynx Preservation

The optimal treatment of patients with advanced laryngeal cancer remains controversial. Standard treatment options have included laryngectomy with or without radiation (RT) and radiation alone with surgical salvage (RTSS). In the United States, total laryngectomy has been the mainstay of treatment. RTSS is generally discouraged as historical data suggest a 15-20% lower survival rate when compared with surgery.

Because of the significant functional morbidity associated with laryngectomy, the Department of Veterans Affair Cooperative Studies Program completed a randomized, prospective study of 332 patients that compared a new organ preservation treatment strategy to conventional laryngectomy and radiation [1]. The experimental treatment arm involved using multiple cycles of neoadjuvant chemotherapy to select patients who had partial (>50%) clinical tumor response for definitive radiation. Thirty percent of patients achieved histologic complete response (CR) after two cycles of chemotherapy. Laryngectomy was reserved for non-responders to the initial chemotherapy and for patients who developed local recurrence following definitive treatment. Final results of this study demonstrated comparable 2, 3, 4, and 10 year survival rates between treatment groups, with successful laryngeal preservation in 66% of the surviving patients randomized to receive neoadjuvant chemotherapy. Analysis of gene expression patterns from this trial indicated that the majority (74%) of patients had tumors with high expression of Bcl-xL. Additionally, successful larynx preservation was significantly associated with low expression of Bcl-xL, particularly among those with low p53 expression [2], [3].

These studies led to a dramatic re-assessment of our treatment approach for advanced laryngeal and hypopharyngeal cancer patients, who face total laryngectomy. Subsequent trials at other institutions have confirmed these results [4],[5]. At the University of Michigan, a retrospective analysis of the results of pilot induction chemotherapy regimens and a detailed analysis of the VA results indicated that a rapid response of a tumor (>50% reduction) to a single cycle of neoadjuvant chemotherapy was predictive of successful larynx preservation in 90% of patients [6]. Additionally, Grossi et al. have found that initial response to chemotherapy was predictive of ultimate response in 86% of patients [7]. Based on these results, a new treatment approach for bioselection was designed and tested in a Phase II study at the University of Michigan (UMCC 9520). [8] In this study, 97 patients were treated with one cycle of induction chemotherapy. Seventy-five percent had a >50% reduction of tumor and were treated with chemo-RT (cisplatin 100 mg/m^2 x 3 cycles), and 20% were non-responders who underwent total laryngectomy followed by RT. Nine percent had surgical salvage following chemoradiotherapy. The overall 3-year survival rate was 85%, the cause-specific survival was 87%, and the larynx preservation rate was 70%. Overall and cause-specific survival was similar in both chemotherapy/radiation and surgically treated patients indicating that appropriate therapy selection can lead to improved survival rates. These represent the highest survival rates ever reported in patients with advanced laryngeal cancer.

While it is not possible to conclude a true survival advantage from a phase II trial, these results (UMCC 9520) were also promising when compared with the results reported from two other large multi-institutional trials. For example, RTOG 91-11 evaluated three treatment strategies in patients with advanced SCC of the larynx (T4 patients were excluded) [5]. Patients were randomized to receive either concurrent cisplatin (100 mg/m2) with RT, induction chemotherapy with cisplatin and 5-fluorouracil followed by RT, or RT alone. The 2-year laryngectomy-free survival rate from the best arm (RT concurrent with cisplatin) in RTOG 91-11 Intergroup Study was 66% similar to 63% at 2 years in UMMC 9520. The overall survival rate of 88% at 2 years in UMCC 9520, however, is better than the best arm of 91-11 (74%) and better than the VA larynx trial (54%). This improvement in results is observed in UMCC 9520 despite the fact that a higher percentage of patients on the Michigan study had stage T4 tumors and tumors arising from the supraglottic larynx, both of which are associated with poorer survival rates.

In trials of concurrent chemoradiation, improved organ preservation outcome has been achieved at a price of enhanced toxicity. In the Intergroup study (RTOG 91-11), the rate of grade 3 and 4 acute mucositis was 20% and 24% in the induction chemotherapy followed with radiotherapy and the radiotherapy alone arms, respectively, and was doubled in the concurrent chemo-RT arm (43%). Also, more deaths that may have been related to therapy were reported in the concurrent chemo-RT arm (5%, compared with 3% in each of the other arms). Enhanced toxicity, primarily mucositis and late dysphagia, is well documented in patients receiving concomitant chemo-RT. [9], [10], [11] It has recently been recognized that mucositis and dysphagia are the main barriers to overcome when treating head and neck cancer patients with cytotoxic chemotherapy and RT [12].

1.2 The Bcl-2 Family

Apoptosis is caused by activation of intracellular proteases, known as caspases, which are directly or indirectly responsible for the morphological and biochemical events that characterize the apoptotic cell. Defects in the regulation of apoptosis make important contributions to the pathogenesis and progression of most cancers including solid tumors, leukemia and lymphomas, and have also been associated with resistance to chemotherapy, radiotherapy, hormonal therapy, and immune-based treatments. Numerous proteins that regulate these cell death proteases have been discovered, including proteins belonging to the Bcl-2 family[13].

Members of the Bcl-2 family play a major role in the pathobiology of head and neck cancer [14], [15]. The use of low-molecular-weight compounds to inhibit Bcl-2 and Bcl-XL function is a novel method with several potential therapeutic advantages. Small molecule inhibitors have the benefit of being more stable with better bioavailability as compared with other biologic and peptide inhibitors. They also may have the ability to penetrate the central nervous system blood brain barrier and do not require a central venous catheter for administration [16] The search for small molecule inhibitors of Bcl-2 and Bcl-XL became possible with the understanding of the detailed atomic structure of both the pro-apoptotic and anti-apoptotic members. Both Bcl-2 and Bcl-XL bind, through their BH3 binding pockets, to the BH3 domains on pro-apoptotic members, an essential step in the execution of the anti-apoptotic signal. The BH3 domains of these pro-apoptotic members are also required for implementing the apoptotic signal [16], [17].

Based on these findings, non-peptide small molecules can be designed to compete with the pro-apoptotic members for binding to the BH3 binding pocket and subsequently disrupting the function of the anti-apoptotic molecules. An excellent review by Wang et al. describes several of these small-molecule inhibitors of Bcl-2 and Bcl-XL and illustrates the various approaches entailed in discovering them [16].

We have retrospectively analyzed biomarkers predictive of successful organ preservation in specimens from the randomized VA Laryngeal Cancer trial and from UMCC 9520. We found that low tumor expression of anti-apoptotic Bcl-xL and overexpression of p53 were significant predictors of success [2], [3], [18], [19]. Over 70% of laryngeal cancer patients show increased expression of Bcl-xL. However, in those with low or absent expression, over 90% (10 of 11 patients) enjoy successful larynx preservation. *In vitro* data on cisplatin sensitivity of head and neck cancer cell lines and *in vivo* animal model studies have indicated that inhibition of Bcl-2/Bcl-xL function by a small molecule BH3 mimetic (AT-101) can increase platinum sensitivity [20], [21], [22], [23].

1.3 AT-101

AT-101 was developed by Ascenta Therapeutics, Inc. for the treatment of patients with advanced cancer. AT-101 is the levorotatory enantiomer of gossypol acetic acid, R-(-)-gossypol acetic acid. Through structure-based computational screening, Ascenta scientists discovered that gossypol is a potent, small-molecule inhibitor targeting the BH3 domain of Bcl-2/Bcl-XL proteins. Subsequently, AT-101 was found to bind to and inhibit the anti-apoptotic (pro-survival) function of Mcl-1 with similar potency to its inhibition of Bcl-2 and Bcl-XL (Yang, 2005). Therefore, AT-101 is appropriately described as a "pan" Bcl-2 inhibitor, by virtue of its similar effects against Bcl-2, Bcl-XL, and Mcl-1—the three Bcl-2 family proteins that promote cancer cell survival—as well as against Bcl-w, another pro-survival member of the Bcl-2 family.

Ascenta is no longer manufacturing AT-101; it is currently manufactured and supplied by Ascentage Pharma Group Corporation (APGC) via a co-development collaboration with Ascenta.

Studies by Ascenta and its collaborators have further demonstrated that:

• AT-101 is more potent than S-(+)-gossypol or racemic gossypol at inhibiting proliferation and inducing apoptosis in cancer cell lines *in vitro*. For example, in the National Cancer Institute (NCI) *in vitro* screen of 60 cell lines from multiple human tumors the average 50% growth inhibition (GI50) value for R-(-)-gossypol was 0.57 μM, whereas the GI50 value was 2.1 μM for racemic gossypol and 14.5 μM for S-(+)-gossypol. R-(-)-Gossypol induced apoptosis in a dose dependent manner in cancer cells that over-express Bcl-2 or Bcl-XL, but has little effect on normal cells with low Bcl-XL and Bcl-2 expression. The ability of R-(-)-gossypol to induce apoptosis in cancer cells is correlated with the level of Bcl-XL; apoptosis is induced via caspase activation. R-(-)-gossypol's anti-proliferative activity is correlated with target cell Bcl-XL/Bcl-XS expression ratios.
• AT-101 acts synergistically with standard chemotherapeutic agents and radiation in inhibiting cancer cell proliferation *in vitro* and *in vivo* tumor models.

• AT-101 has *in vivo* tumor growth inhibition activity as a single agent or in combination in with docetaxel, cisplatin, CHOP (cyclophosphamide, doxorubicin, Oncovin, and prednisone) or radiation in human tumor xenograft models. The xenograft models tested include A549 (NSCLC), PC-3 (prostate), MDA-MB-231 (breast) and others.
• More recent studies have also indicated that AT-101 treatment results in potent induction of the natural ligands of Mcl-1, Bcl-2 and Bcl-xL, Noxa and Puma, which are pro-apoptotic, as well as inhibiting angiogenesis [24], [25].

1.3.1 Pre-clinical Models with AT-101 in SCCHN:

Data from the University of Michigan Comprehensive Cancer Center [26] have shown that Bcl-2 orchestrates a cross talk between tumor cells and endothelial cells that have a direct effect on the progression of HNSCC. Notably, Bcl-2 is significantly up-regulated in the tumor associated endothelial cells compared with the endothelial cells of normal oral mucosa in patients with HNSCC. In this investigation, the effect of AT-101, a small-molecule inhibitor of Bcl-2, was evaluated in conjunction with the cell cycle and survival of endothelial cells and HNSCC and on the progression of xenografted tumors. The combination of AT-101 and cisplatin showed enhanced cytotoxic effects for endothelial cells and HNSCC in vitro, compared with single drug treatment. Whereas cisplatin led to an expected G2-M cell cycle arrest, AT-101 mediated an S-phase cell cycle arrest in endothelial cells and in HNSCC. In vivo, AT-101 inhibited tumor angiogenesis and induced tumor apoptosis without significant systemic toxicities. Combination of AT-101 and cisplatin enhanced the time to tumor failure (i.e., 4-fold increase in tumor volume), compared with either drug given separately. Collectively, these data reveal that therapeutic inhibition of Bcl-2 function with AT-101 is sufficient to arrest endothelial cells and HNSCC in the S phase of the cell cycle and to inhibit head and neck tumor angiogenesis.

1.3.2 Clinical Experience with AT-101 in Solid Tumors

1.3.2.1 AT-101 in Combination with Docetaxel in NSCLC

Clinical trials conducted with AT-101 are ongoing. Data in non-small cell lung cancer presented at the 2009 American Society Clinical Oncology Annual Meeting [27] demonstrated improvements in progression-free and overall survival when AT-101 (40 mg b.i.d. days 1-3) was administered with docetaxel (75 mg/m^2 every 21 days). Additionally, a biomarker of AT-101 activity was developed to identify a genomic predictor of sensitivity to AT-101. An analysis of gene expression data by Bayesian regression revealed a 500 gene predictor of sensitivity to AT-101 that was cross validated in a leave one out analysis and in an independent cohort of 32 NSCLC cell lines.

1.3.2.2 AT-101 in Combination with Docetaxel and Prednisone in Prostate Cancer

A Phase 1/ 2 trial of AT-101, using a higher dose of AT-101 given for a shorter period of time (denoted as a pulse dose schedule) in combination with docetaxel and prednisone in hormone refractory prostate cancer (HRPC) has recently been published [28]. During the Phase 1 portion of this study, patients were enrolled in successive cohorts of 3 patients with doses of AT-101 of 40-60 mg BID x 3 days on days 1-3 in combination with docetaxel 75 mg/m2 on day 1, every 21

days. No dose limiting toxicities (DLTs) were observed and dose escalation was not increased beyond 60 mg BID x 3 days, as the dose of 80 mg BID x 3 days every other week was associated with ileus in a separate study.

Three cohorts of patients were treated with the longest treatment duration being 12 cycles of therapy in one patient. All patients treated had decreases in PSA levels. All patients with chemotherapy-naïve HRPC had at least a 30% decrease in PSA and 75% of patients met the criteria for a PSA partial response (e.g. ≥ 50% decrease). Partial responses per RECIST were also observed.

In the Phase 2 portion of the trial, patients were enrolled into 2 cohorts: chemotherapy naïve HRPC (cohort A) and docetaxel-refractory HRPC (cohort B). 40 mg of AT-101 was administered b.i.d. on days 1-3 in combination with docetaxel and prednisone. Thirty six (36) patients were enrolled into cohort A. AT-101 was well tolerated and the majority of Adverse Events (AEs) were Grade 1 or 2. There were no notable differences in the rates or severities of fatigue, gastrointestinal toxicities (e.g. nausea, vomiting, and diarrhea), and grade 3 or 4 cytopenia compared to historical controls. Thirty six (36) percent of patients received 10 cycles of therapy and there were no cases of non-mechanical small bowel obstruction. Efficacy data indicate that AT-101 has biological activity in combination with docetaxel and prednisone in men with HRPC:

- 72% of patients achieved a 30% decrease in PSA.
- 67% of patients achieved a PSA partial response (Bubley Criteria).
- 45% of patients with measurable diseases achieved a partial

  responses per RECIST.

Forty (40) patients were enrolled into cohort B. AT-101 was well tolerated and 35% of patients received at least 4 months of therapy. One of 40 patients reported non-mechanical small bowel obstruction. Efficacy data indicate that AT-101 has biological activity in combination with docetaxel and prednisone in men with docetaxel-refractory CRPC:

- 41% of patients achieved a 30% decrease in PSA.
- 22% of patients achieved PSA partial responses (Bubley Criteria).
- 24% of patients achieved partial responses per RECIST.

Ascenta conducted a randomized phase 2 study of AT-101 or placebo in combination with docetaxel and prednisone in 221 men with chemotherapy naïve HRPC. This study opened for enrollment in October 2007 and was completed in April 2009. Patients are randomly assigned to receive either Arm A: oral AT-101 (40 mg [4 tablets of 10 mg] b.i.d. on cycle days 1-3), with docetaxel (75 mg/m2 intravenously over 1 hour every 21 days [one cycle]), and oral prednisone (5 mg b.i.d. on days 1-21), or Arm B: docetaxel (75 mg/m2 intravenously over 1 hour every 21 days [one cycle]), oral prednisone (5 mg b.i.d. on days 1-21), and oral placebo (4 tablets b.i.d. on cycle days 1-3). Randomization are stratified by ECOG performance status (0 or 1 versus 2) and baseline pain (absent versus present).

In general, the treatment was well tolerated, with a median number of cycles administered of approximately 9 cycles in each arm of the study. The majority of AEs of grade 1, 2, or 3 were similar between treatment arms. There were more grade 3 or 4 AEs on the AT-101 arm of the

study although only fatigue was statistically significant. There were more patients who discontinued study treatment due to an adverse event on the AT-101 arm of the study and this was primarily due to grade 1 or 2 neuropathy. There were more reports of pulmonary embolism on the AT-101 arm of the study compared to the placebo arm (6 vs. 2, respectively).

After 108 deaths, an overall survival (OS) analysis was performed and demonstrated no significant improvement in OS between treatment arms. There were trends favoring the AT-101 arm in PSA, RECIST and PFS, but they did not reach statistical significance in this moderately sized Phase 2B trial.

A subgroup analysis of treatment effect using the TAX327 study-defined risk groups (based on baseline anemia, visceral disease, bone progression at baseline, and baseline pain) was performed. A difference between arms, favoring AT-101 treatment, was demonstrated in the high-risk group (N=32 patients). This difference was consistent across efficacy parameters and the improvement in OS was 5 months (14 v. 19 months).

1.3.2.3 A Phase 1 Study of AT-101 in Combination with Paclitaxel and Carboplatin in Solid Tumors

AT-101 is being investigated in combination with paclitaxel and carboplatinum in a Phase 1 study being conducted by the NCI. AT-101 is dosed 30-40 mg b.i.d. on days 1-3 in combination with paclitaxel and carboplatinum on day 1 on a 21 day cycle. 22 patients have been evaluated, including 8 patients treated with AT-101 40 mg, paclitaxel 175 mg and carboplatin AUC 6. Thus far, the treatment is well tolerated.

1.4 CT with perfusion in locally advanced head and neck cancer

CT perfusion (CTP) provides the unique ability to noninvasively quantify the microvascular blood flow of tumors and normal tissues. CTP provides important information on the biologic response to non-surgical organ preservation and has the potential to determine whether the tumor is properly responding to NSOPT. The ability to non-invasively monitor tumor response during non-surgical organ preservation therapy (NSOPT) is desirable in cancer treatment. Accurate identification of non-responding tumors would permit early implementation of adaptive radiotherapy, adjuvant chemotherapy or surgical resection. This would have widespread implications and increase patient survival, create a rational approach for implementation for costly new chemotherapy agents and help reduce the growing costs of salvage therapy and end of life care.

The initial results investigating the role CTP for monitoring response to NSOPT are promising. These results suggest that changes in CTP have the ability to non-invasively predict response to NSOPT. [29], [30], [31], [32], [33]. There is also early evidence to suggest that CTP is correlated with molecular biomarkers that may increase the efficacy of targeted chemotherapy. Recent reports indicate new data suggesting that CT perfusion is predictive of the expression of the epidermal growth factor receptor (EGFR) which is the mechanism of the drug cetuximab and a correlation between CT perfusion and interleukin-8 [34].

The most encouraging aspect of such investigations is that there is now consensus among radiation oncologists that biologic imaging response precedes changes in tumor volume for predicting response. The early data from the University of Michigan shows that the CTP parameters vary during the course of treatment but blood flow and blood volume tend to be reduced in responders after approximately 4000Gy. However, there is no clear consensus and more investigations need to be performed .

1.5 Study Hypothesis and Rationale

Treatment of patients with one cycle of induction chemotherapy to select for organ preservation of the larynx has become our standard approach at the University of Michigan. When compared to historical controls, our data from UMCC 9520 appears to have improved survival outcomes, which led us to believe that some patients are better suited to undergo early surgical salvage. Of those treated with organ preservation, however, long-term toxicities remain barriers to successful outcomes. Hence, we propose to study patients with a similar treatment strategy (i.e. one cycle of induction chemotherapy followed by chemoradiation for those responding to chemotherapy or surgical salvage for those failing to respond) with changes to the treatment regimen which will hopefully allow for increased organ preservation rates and reduced toxicity from induction chemotherapy and chemoradiotherapy. Several randomized trials of induction chemotherapy have demonstrated that the combination of a taxane and platinum agent achieves response rates equal or better than our standard cisplatin and 5-fluorouracil [35], [36] and improvements in overall survival with less toxicity have been noted. The most significant and consistent biomarker of successful organ preservation and survival has been response to induction chemotherapy.

While we have effectively used this response as a biologic indicator to select patients for either surgery or an organ preservation approach, the next step is to find substitute biomarkers and to increase complete response rates in order to improve on our 60-70% organ preservation rates. In the original VA Larynx trial, we identified p53 expression and Bcl-xL expression as the most useful biomarkers for organ preservation. We confirmed these findings in our Phase II trial, UMCC 9520 and extended the findings to improved overall survival in patients with low Bcl-xL. We have investigated the effects of Bcl-xL gene expression and p53 mutation in cell lines that are sensitive and resistant to cisplatin and confirmed that sensitivity can be restored by inhibition of Bcl-xL using the small molecule inhibitor, AT-101[20], [21], [22]. We hypothesize that the combination of docetaxel and cisplatin will be less toxic than cisplatin/5-fluroruracil as induction therapy and that the addition of AT-101 will increase the clinical complete response rate by inhibiting Bcl-xL in treated patients. An increase in CR rate will allow more patients to be effectively treated for organ preservation.

Published data demonstrate equal efficacy and improved quality of life when platinum and a taxane were compared with platinum and 5-Fluorouracil [37]. Additionally, weekly cisplatin regimens (30-40 mg/m^2) with radiotherapy appear to be equally efficacious and better tolerated than standard high-dose cisplatin (100 mg/ m^2) regimens with radiation therapy for locally

advanced SCCHN [38] We will thus attempt to reduce toxicity from induction chemotherapy with the use of docetaxel/cisplatin (or carboplatin) (TP) in place of our previously used standard regimen of cisplatin and 5-fluorouracil (PF) and administer weekly cisplatin (or carboplatin) with radiation for those patients who are responders to induction therapy. Finally, Phase I/II testing of the small molecule inhibitor, AT-101, has recently been completed, and suggests activity in solid tumors when combined with cytotoxic agents. Since we have achieved such high survival rates with our treatment selection approach in laryngeal cancer, our ultimate goal is to reduce the rate of salvage laryngectomy which should improve quality of life. We hypothesize that specific inhibition of Bcl-2/Bcl-xL function can increase response rates to neoadjuvant chemotherapy and decrease the need for salvage laryngectomy. We also hypothesize that the addition of a second cycle of induction chemotherapy will improve response and decrease need for salvage laryngectomy. Hence, we propose this study: the treatment of patients with advanced SCC of the larynx with one-two cycles of platinum plus docetaxel with AT-101, followed by chemoradiotherapy for those responding to this induction regimen and reserving total laryngectomy for those who are non-responders.

1.6 Quality of Life

The overall goal of this treatment approach is to optimize patient selection in order to decrease the need for protracted multi-modality, highly morbid treatment (surgery and radiation) and to eliminate therapeutic redundancy by identifying those patients who are unlikely to be responders to either chemotherapy or radiation therapy. We expect to demonstrate a significant improvement in voice-related quality of life (V-RQOL) compared to patients in our prior (UMCC 9520) protocol [39]. Given the change in chemotherapy regimens throughout our proposed trial, we hypothesize that QOL scores will be improved in this evaluation. Hence, we plan to evaluate physical and social well-being with the FACT-HN and speech and swallowing with the University of Michigan HN-QOL tool [40], [41].

2.0 Objectives

2.1 Primary

2.1.1 To determine the salvage surgery rates of this treatment paradigm compared to the salvage surgery rates of patients treated on UMCC 9520.

2.1.2 To estimate the overall response rate to induction chemotherapy with platinum and docetaxel plus AT-101 following one and/or two cycles in patients with advanced laryngeal cancer.

2.1.3 To determine the progression-free survival rates of this treatment paradigm compared to the progression-free survival rates of patients treated on UMCC 9520.

2.1.4 To determine toxicities of induction chemotherapy plus AT-101 and chemoradiation in patients on this treatment .

2.2 Secondary

2.2.1 To determine expression of Bcl2/BclxL, EGFR and p53 in pre and posttreatment biopsy specimens

2.2.2 To compare the overall response rates of the 1st cycle of induction chemotherapy with platinum and docetaxel to platinum and docetaxel plus AT-101.

2.2.3 To compare the overall response rates of induction chemotherapy with platinum and docetaxel plus AT-101 to the rates of induction chemotherapy with cisplatin and 5-fluorouracil from the historical group treated on UMCC 9520.

2.2.4 To estimate overall and cause-specific survival rates in patients on this treatment.

2.2.5 To evaluate the quality of life (QOL) of this treatment paradigm

2.2.6 To evaluate tumor vascular perfusion in response to induction chemotherapy utilizing CT with perfusion.

3.0 Eligibility Requirements

3.1 Patients must have pathologically confirmed, previously untreated, resectable, squamous cell carcinoma of the larynx or hypopharynx.

3.2 Disease must be Stage III or IV.

3.3 Tumor must be potentially surgically resectable and curable with conventional surgery and radiation therapy.

3.4 Patients must undergo pre-treatment DL endoscopic tumor staging and CT scanning.

3.5 ECOG Performance status 0-1 (See **Appendix A**).

3.6 Pre-treatment laboratory criteria:

3.6.1 WBC $\geq$ 3500/ul, granulocyte $\geq$ 1500/ul.

3.6.2 Platelet count $\geq$ 100,000/ul.

3.6.3 Calculated or measured creatinine clearance $\geq$ 60 cc/min for cisplatin candidates, $\geq$ 30 cc/min for carboplatin candidates.

3.6.4 Total Bilirubin $\leq$ 1.5 X ULN.

3.6.5 AST and ALT $\leq$ 2.5 X ULN.

3.7 Patients must give documented informed consent to participate in this study.

4.0 Exclusion Criteria

4.1 History of secondary malignancy or history of other malignancy within the last three years (patients who have been disease-free for three years, or have a history of completely resected non-melanoma skin cancer or successfully treated in situ carcinoma are eligible).

4.2 Prior head and neck radiation or prior chemotherapy.

4.3 Documented evidence of distant metastases.

4.4 Active infection.

4.5 Pregnancy or lactation. Patients must agree to use adequate contraception (hormonal or barrier method of birth control) prior to study entry, for the duration of study participation and for 3 months after discontinuing therapy.

4.6 Any medical or psychiatric illness which in the opinion of the principal investigator would compromise the patient's ability to tolerate this treatment.

4.7 Patients residing in prison.

4.8 Age < 18 years.

4.9 Patients with psychiatric/social situations that would limit compliance with study requirements are not eligible.

4.10 Patients with prior radiation to the head and neck..

4.11 Patients with Grade $\geq$ 2 peripheral neuropathy.

4.12 Any history of severe hypersensitivity reaction to docetaxel or other drugs formulated with polysorbate 80.

4.13 Class 3 or 4 cardiac disease as defined by the New York Heart Association Functional Classification (Appendix B).

4.14 Unstable angina or a history of myocardial ischemia within prior 6 months

4.15 Malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel, ulcerative colitis, inflammatory bowel disease, partial or complete small bowel obstruction.
4.16 Prior use of gossypol or AT-101, or known hypersensitivity to gossypol or AT-101.
4.17 Patients taking any other concurrent approved or investigational anti-cancer therapy (e.g. chemotherapy, immunotherapy, targeted or biologic therapy).

5.0 Initial Clinical Evaluation

Note: All Initial Clinical Evaluations must occur prior to Day 1

5.1 Prior to day 1:Complete history and physical examination, multidisciplinary examination by Otolaryngology, Radiation Oncology, and Medical Oncology, with descriptive documentation of extent of primary tumor and regional disease.

5.2 Satisfactory biopsy of the primary tumor.

5.3 Representative tumor specimen sent to Head and Neck Cancer Research Laboratory for ancillary studies.

5.4 Pre-treatment blood sample (24 cc to Dr. Carey; 2 - 6mL red tops and 2 – 6mL green tops). The blood serum will be stored for tumor marker analysis and to create lymphoblastoid cell lines for harvesting normal DNA.

5.5 Complete dental evaluation, if required as per radiation oncologist

5.6 Completion of laboratory studies: Comprehensive panel, CBC with differential and platelets, magnesium and creatinine clearance (calculated or measured).

5.7 Diagnostic CT scan of the neck with perfusion. (CT scan with perfusion is the same radiation exposure and risk as a standard CT scan.)

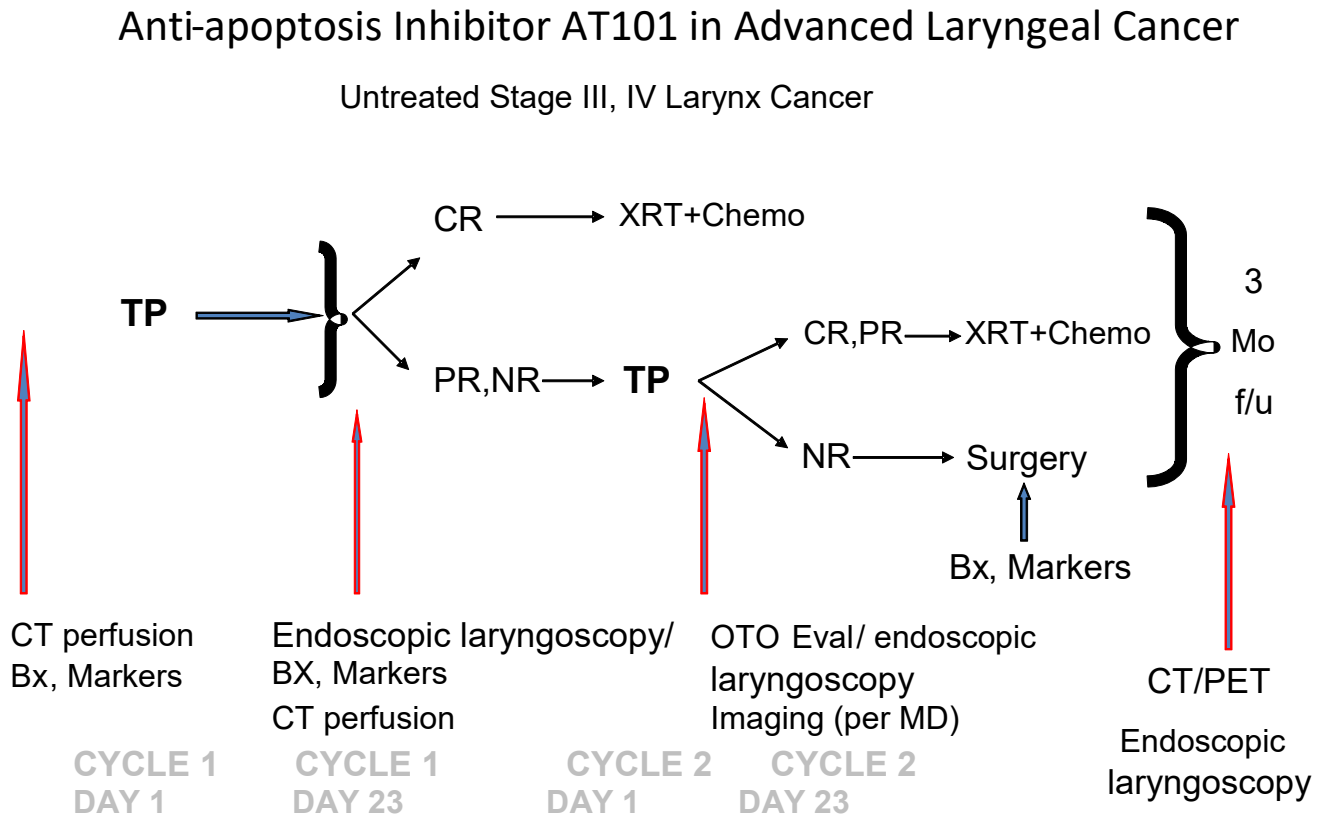
5.8 Chest X-Ray or diagnostic CT of chest/thorax, as determined by the treating physicians. (CT/PET may be used as an alternative to the diagnostic chest CT at the discretion of the investigator).

5.9 CT of the abdomen, if clinically warranted (as deemed necessary by the treating physicians).

5.10 Bone scan, if clinically warranted (as deemed necessary by the treating physicians).

5.11 Audiograms for patients with hearing complaints or hearing loss at the discretion of the prescribing physician.
5.12 Quality of life assessments (FACT-HN: version 4, HN-QOL, AND V-RQOL)

6.0 Schema



TP = docetaxel /cisplatin (carboplatin);

Days in this schema are approximate only; see Calendar, Section 9.0.

7.0 Study Design

7.1 Chemotherapy/Radiation/Surgery schedule (see schema section 6.0)

NOTE: Carboplatin may be substituted for cisplatin at any time if patients are not candidates for cisplatin or are not able to tolerate cisplatin secondary to toxicity, as deemed necessary by the patient's prescribing medical oncologist.

7.1.1 Following enrollment, patients will be randomized to one of two arms of induction chemotherapy (in a 2:1 ratio): TP + AT-101 **or** TP alone (see Schema, Section 6.0).

7.1.2 **(Both Arms) Day #1:** Patients will undergo induction chemotherapy with (TP) docetaxel (Taxotere) 75 mg/m^2 and cisplatin 100 mg/m^2 (or Carboplatin AUC 6 with max dose 700 mg).

7.1.3 **(AT-101 Arm):** On day 1, patients will initiate AT-101 40 mg orally every 12 hours (+/- 1 hour) for a total of 6 doses.

7.1.4 Cycle 1 **Day #23 (+/- 3 days):** Patients will undergo an endoscopic laryngoscopy with biopsy. Patients will also undergo a repeat CT scan of the neck with perfusion within a week (+/- 7 days) of their perspective biopsies.

7.1.5 Patients with a > 50% but < 100% response (PR) or no response (NR) to induction chemotherapy will undergo a repeat cycle of TP and AT-101 (as outlined in sections 7.1.1-7.1.3).

Cycle 2 Day #23 (+/- 3 days): Patients will have radiographic imaging per investigator/MD discretion, modality of choice; OTO clinical evaluation and endoscopic laryngoscopy by otolaryngology

7.1.6 **Treatment with Chemoradiotherapy:**

After induction chemotherapy, patients with ≥ 50% response or 100% response (PR, CR) following clinical evaluation (Cycle 2 Day 23 +/- 3 days) will undergo treatment with RT (Total dose 70 Gy) in combination with weekly Cisplatin (50 mg/m^2) or Carboplatin (AUC 2.5 with max dose 230 mg). The tumor dose may be exceeded by <10% in protocol subjects based on clinical judgement.

Twelve weeks following the completion of RT, patients will undergo a repeat endoscopic laryngoscopy and PET imaging at the discretion of the prescribing physicians. The primary tumor site will be biopsied if clinically indicated. Neck nodes that are persistent, clinically suspicious nodes, including PET positive nodes, will be excised.

Patients with positive laryngeal biopsies following chemoradiation will undergo salvage laryngectomy. If the laryngeal biopsy is negative but clinically or PET positive neck lymph nodes remain, patients will undergo neck nodal dissection alone. Patients with PET negative necks and no clinically suspicious adenopathy may have the neck observed.

7.1.7 **Early Salvage Laryngectomy:** Those patients who remain NR after 2 cycles

of induction chemotherapy, will be treated with salvage laryngectomy followed by RT. Cisplatin or Carboplatin will be added to radiation for patients whose surgical pathology reveals high-risk features (i.e. extracapsular spread, > 2 positive lymph nodes, perineural invasion, or positive margins.) Cisplatin will either be dosed a 100 mg/m^2 (or Carboplatin AUC 6 with max dose 700 mg) every 21 days or 50 mg/m^2 (or Carboplatin AUC 2.5 with max dose 230 mg) weekly at the discretion of the prescribing physician. See section 7.2 for prescribing details for cisplatin.

7.1.8 **Late Salvage Laryngectomy:** Once patients are disease-free following chemoradiation and nodal neck dissections (if needed), patients will be followed at regular intervals as prescribed by their treating surgeon. Patients whose physical examination or whose imaging studies are concerning for recurrent disease will undergo biopsy. If positive, they will undergo salvage laryngectomy.

7.2 Chemotherapy Administration with Cisplatin (Carboplatin), and Docetaxel

7.2.1 **Docetaxel (Taxotere):** Docetaxel 75 mg/m^2 administered as an IV infusion over one hour on **day #1.**

7.2.2 With Docetaxel administration, all patients will receive dexamethasone 8 mg PO BID for 3 consecutive days, starting 1 day before docetaxel administration. Dexamethasone 20 mg IV (x1) may be administered in place of oral dexamethasone as deemed necessary by the prescribing physician.

7.2.3 **Cisplatin:** Cisplatin 100 mg/m^{2,} administered as an IV infusion will run over one hour on **day #1** following docetaxel.

7.2.4 An aggressive antiemetic regimen is required before the administration of cisplatin, consisting of a neurokinin 1 antagonist, sertotonin (5-HT3) antagonist and a steroid (dexamethasone).

7.2.5 Pre-cisplatin hydration: normal saline plus 1 gram/L Magnesium Sulfate plus KCl 20 mEq/L i.v. infusion at 500 mL/hr x 3 hours before cisplatin. Alternative pre-hydration regimens may also be administered at the discretion of the prescribing physicians.

7.2.6 Mannitol 12.5 gm i.v. bolus may be given immediately prior to cisplatin, followed by 25 gm of mannitol in 500 mL of normal saline with cisplatin as deemed necessary.

7.2.7 Post-hydration: Normal saline plus KCl 20 mEq/L plus $MgS0_4$ 1 gm/L at 500 mL/hr x 3 hours. Alternative post-hydration regimens may also be administered at the discretion of the prescribing physicians.

7.3 Evaluation of Response to Treatment

7.3.1 Careful evaluation of tumor extent will be separately recorded for the primary tumor and regional nodes at specified intervals. These will be based on laryngoscopies performed pre-treatment, Day 23 (+/- 3 days) after start of induction chemotherapy and AT 101, and 12 weeks after completion of chemoradiation, if clinically indicated. Standardized response criteria will be used. CT scans will be used at the discretion of the clinician to

supplement clinical exams. However, it is not necessary for imaging abnormalities to revert to normal in order for a patient to be considered a clinical CR.

7.3.2 Open biopsy of any clinically persistent or PET positive neck nodes is required at the 12 week post chemoradiation. Patients with any nodes initially > 3 cm in size who are PET positive at 12 weeks post chemo-RT will undergo neck dissection. Patients whose PET shows a CR at 12 weeks post chemo-RT, will undergo clinical observation.

7.3.3 For post treatment evaluations, diagnostic CT scans and/or PET scans will be obtained prior to scheduled endoscopies for tumor assessment.

7.3.4 Outpatient clinical examinations will be performed at the completion of radiation therapy, and for 3 years with timing of evaluations at the discretion of the treating physician. Clinical examinations will be performed by one, but not all three, of the following disciplines: medical oncology, radiation oncology, or surgical oncology. To meet the examination requirements, patients need to be seen by at least one discipline (but not all three) during their followup up period.

8. 0 Dose Delays and Treatment Modifications

Toxicity	Action Taken
Abdominal Pain/Nausea/Vomiting	See Section 8.1 of the Protocol
• Total bilirubin is > ULN • AST and/or ALT is > 2.5 x ULN • Alkaline phosphatase ≥ 2.5 x ULN *and* AST and ALT is > 1.5 x ULN	**Step 1:** Interrupt treatment (AT-101 + TP) or (weekly platinum) until the toxicity has resolved, up to 2 weeks (14 days). **Step 2: For AT-101 + TP:** Restart treatment with TP and AT-101. Docetaxel should be reduced by 15 mg/m^2 and cisplatin by 20 mg/m2 and carboplatin by an AUC 1 for the 1st and 2nd events and AT-101 should be reduced to 30 mg b.i.d. **Step 2: For Weekly Platinum:** Cisplatin should be reduced by 10 mg/m^2 and Carboplatin by an AUC 0.5. Monitor as clinically indicated.
CrCl < 60: Discontinue cisplatin, change to carboplatin if CrCl ≥ 30 If CrCl < 30, hold carboplatin. When CrCl returns to ≥ 30, initiate/resume carboplatin at previous dose.	
Tinnitus: if observed on cisplatin, may change to carboplatin at the discretion of the prescribing MD	
Hematologic Toxicity	
• Neutrophil count is < 1000 cells/ mm^3 • Platelet count is < 75,000 cells/mm^3	**Step 1:** Interrupt treatment (AT-101 and TP) or (weekly platinum) until the toxicity has resolved to ≤ Grade 1 or pre-therapy baseline, up to 2 weeks (14 days). **Step 2:** Restart treatment with TP and AT-101 or weekly platinum; monitor as clinically indicated.

• Grade 4 neutropenia (< 500 cells/mm^3) lasting 7 days or more • Grade 3 or 4 neutropenia with an oral temperature of at least 38.5°C	**Step 1:** Interrupt treatment with (TP and AT-101) or (weekly platinum) until resolved to ≤ Grade 1, up to 2 weeks (14 days). **Step 2: For TP and AT-101:** Restart treatment with TP and AT-101. Docetaxel should be reduced by 15 mg/m^2 , cisplatin by 20 mg/m2, carboplatin by an AUC 1for the 1st and 2nd events and AT-101 should be reduced to 30 mg b.i.d. **Step 2: For Weekly Platinum:** Cisplatin reduced by 10 mg/m^2 and carboplatin by AUC 0.5. Monitor as clinically indicated.
Other Toxicities	
• Any Grade 2 or 3, if clinically significant **with the exception of mucositis (Grade 2 and 3 mucositis is expected with chemoRT)**	**Step 1:** Interrupt treatment of (AT-101and TP) or (weekly platinum) up to 2 weeks (14 days), until toxicity resolves to ≤ Grade 1. **Step 2: For TP and AT-101:** Restart treatment with TP and AT-101. Docetaxel should be reduced by 15 mg/m^2 , cisplatin by 20 mg/m2 and carboplatin by an AUC 1 for the 1st and 2nd events and AT-101 should be reduced to 30 mg b.i.d. **Step 2: For Weekly Platinum:** Cisplatin should be reduced by 10 mg/m^2 and Carboplatin by an AUC 0.5. Monitor as clinically indicated.
• Any recurrent Grade 2 or 3 after two (2) dose reductions, if clinically significant **with the exception of mucositis (see above)**	**For TP and AT-101 OR Weekly Platinum:** Discontinuation of treatment with AT-101 and docetaxel and follow-up per protocol.
• Any Grade 4	Discontinuation of treatment with AT-101and TP OR Weekly Platinum and follow-up per protocol.
See Section 8.1 for additional guidelines	

8.1 Guidelines for Nausea, Vomiting or Abdominal Pain

If a patient has ≥ Grade 2 nausea, ≥ Grade 2 vomiting, or any abdominal pain that lasts for longer than 48 hours, (AT-101 and TP) dosing should be held and the following actions are recommended:

1. Physical examination, including the performance of vital signs
2. Screening abdominal radiography to include an abdominal series to exclude ileus, SBO, or pneumatosis intestinalis
3. Consideration for obtaining a CT scan with contrast of the abdomen should be made based on clinical judgment

If ileus, SBO, or pneumatosis intestinalis are excluded, dosing of AT-101 may be reinstated following the provisions in Section 8.0, Dose Delays and Dose Modifications for Toxicity. If a patient is

determined to have ileus, SBO, or pneumatosis intestinalis, dosing of AT-101 and TP will be held and expectant management with supportive care is recommended, unless clinical signs or symptoms are present that suggest septicemia or abdominal catastrophe that warrants surgical management. No further AT-101 can be administered following recovery from these events.

9.0 Study Calendars

9.1 Induction Chemotherapy (TP and AT-101)

Assessment	Pre-Therapy	C1D1	C1D2	C1D	C1D14 +/- 3 days	C1D23 +/- 3 days	C2D1/ +4 -18 days from C1D23 DL	C2D2/	C2D3/	C2D23 +/- 3 days
H&P*	X									
Medical Oncology Exam	X						X			
Informed Consent	X									
Endoscopic laryngoscopy with Biopsy	X					X[8]				
Dental Eval, if required[2]	X									
CBC with diff	X	X[14]			X[8]		X[8]			
Comp and Mg	X	X[14]					X[8]			
Creatinine Clearance[6]	X									
CXR/chest CT[4]	X									
Neck CT w/ perfusion	X					X[7]				
PET scan[5]	X									
Radiographic Imaging										X[12]
Abdomen CT[1]	X									
Bone scan, if required[2]	X									
EKG	X									
Audiogram, if required[3]	X									
Quality of Life Assessments[10]	X						X			
Biologic Correlatives[11]	X						X[8]			
AT-101		X[13]	X[13]	X[13]			X[9, 13]	X[9, 13]	X[9, 13]	
Chemo (TP)		X					X[9]			
Otolaryngology exam/response assessment										X

*Evaluations by Radiation Oncology, Otolaryngology, and Medical Oncology prior to day 1.

[1] If clinically warranted, as determined by the treating physicians.

[2] If clinically warranted, as determined by the treating physicians.

[3] At the discretion of the prescribing physician for patients with c/o hearing loss.

[4] Either a CXR or a chest CT must be performed. The prescribing physicians will determined which imaging study is most appropriate. (CT/PET may be used as an alternative to the diagnostic chest CT at the discretion of the investigator).[5]PET also repeated at least 12 weeks post completion of concurrent chemoradiation.per the discretion of the prescribing physician

[6] Calculated or measured. Must be $\geq$ 60 cc/min for cisplatin and $\geq$ 30 cc/min for carboplatin.

[7] May be performed (+/-) one week the patient's scheduled biopsy.

[8] May be performed (+/-) 3days if scheduling conflicts arise.
[9] Patients who attain PR/SD will be evaluated on day 27 (+/- 14 days) and will be treated with one additional cycle of TP + AT-101. Patients are permitted to begin subsequent TP outside of window, if medically warranted, as determined by treating physicians prior to TP. Patients will remain in initial randomized arms in subsequent TP.
[10] Quality of life instruments (FACT-HN: version 4, HN-QOL, and V-RQOL) to be administered pre-therapy.
[11] Correlative Blood draw(s): 2 - 6mL red tops and 2 – 6mL green tops.
[12] Imaging and modality will be performed per investigator/MD discretion
[13] Cycle 1, only if randomized to AT101 arm. All patients will receive AT-101 in cycle 2. Diary will be provided for completion w/ AT-101 dispensation.
[14] Ok to use screening labs if obtained within 14 days of day 1.

9.2 CR/PR Study Arm: RT + Weekly Cisplatin/Carboplatin

Assessment	Pre-Therapy	Weekly during RT	12 weeks post RT	Follow Up
Comp and Mg	X	X		
CBC with diff	X	X		
Cisplatin (50mg/m^2) **or** Carboplatin (AUC 2.5 with max dose 230 mg)		X		
PET/CT scan of neck[1]			X	
Endoscopic laryngoscopy with biopsy[5]			X	
Medical Oncology Exam		X		X[2]
Toxicity Evaluation		X[4]		X[2,4]
Surgical Evaluation			X	X[2]
Radiation Oncology Evaluation		X		X[2]
QOL Assessments		X		X[3]

[1] Pet imaging is at the discretion of the prescribing physician. If performed, may be scheduled up to one week prior to scheduled laryngoscopy (in office or OR).
[2] Outpatient clinical examinations will be performed at the completion of radiation therapy and for 3 years with timing of evaluations at the discretion of the treating physician. Clinical examinations must include an evaluation by one, but not all three, of the following disciplines: Medical Oncology, Radiation Oncology, and/or Surgical Oncology. Moreover, it is NOT necessary for the patients to see each discipline during their follow-up period.
[3] Quality of life instruments (FACT-HN: version 4, HN-QOL, and V-RQOL) will be administered pre-therapy, and at 6, 12, and 24 months post cisplatin-RT or surgery-RT (+/- 4 months post RT or surgery RT). See section 21.0.
[4] Toxicities will be evaluated using the CTCAE, version 3.
[5]Biopsies as deemed necessary by the evaluating surgeon.

10.0 Response Assessment Criteria:

10.1 With over 15 years of experience in the clinical evaluation of tumor response after induction chemotherapy, the Head and Neck surgical faculty have arrived at a consistent system of classifying clinical tumor response. The "consistent system" refers to evaluating the primary tumor during endoscopic laryngoscopy; following chemotherapy, the same surgeon will again re-evaluate the product of the dimensions to evaluate for response (see CR and PR below). These criteria have been developed and applied uniformly across the protocols conducted by the Head and Neck Oncology Program. They pertain only to the primary tumor. Standard response criteria for chemotherapy response are used for nodal assessments. **Treatment decisions (i.e. surgery vs Chemo+RT) are based on response of the tumor at the primary site as assessed by the Head and Neck surgeon**

10.2 Tumor responses to chemotherapy or chemoradiation will be determined clinically by the surgeon performing the laryngoscopy.

10.3 Radiologic imaging studies (CT perfusion) will be used in conjunction with the physical examination in determining tumor response to chemotherapy or chemoradiation.

10.4 Clinical CR: No visible or palpable evidence of gross tumor at time of direct laryngoscopy. This does not include vocal cord function or changes in mobility. Visible ulceration, induration or mass lesion is recorded as partial response or stable disease, depending on percent reduction in the product of longest diameter at its perpendicular. In some cases, specific tumors have deeply invasive components for which surface assessment of tumor extent is inaccurate. Pre-treatment assessment is provided by CT scan and a decision to use imaging for response assessment is made prior to beginning protocol treatment. Post-treatment response assessment is determined by comparing pre to post treatment CT scans as appropriate. Specific examples would include some supraglottic cancers with extensive pre-epiglottic space extension, clinical CR would require complete disappearance of pretreatment enhancing mass (other than edema) effect on the post treatment imaging study.

Histologic CR: Primary tumors exhibiting a clinical CR or at least a 90% PR and having a negative post-treatment biopsy are classified as histologic CR. Biopsies are specifically obtained from any masses or suspicious ulceration, induration or from the geographic center of the primary tumor site radiated on pre-treatment tumor diagrams.

Clinical PR: Significant (>50%) reduction in the product of the longest primary tumor dimension and its perpendicular compared to pre-treatment clinical (endoscopic) measurements or imaging study as necessary.

Non-Responders (< PR): A 50% or less reduction in the product of the longest primary tumor dimension and its perpendicular compared to pre-treatment clinical (endoscopic) measurement or imaging study as necessary.

11.0 Criteria for Discontinuation of Treatment

11.1 Unacceptable adverse event(s).

11.2 Intercurrent illness, which prevents further administration of treatment.

11.3 Patient preference.

11.4 Progressive disease.

11.5 Life threatening or other unacceptable drug-related toxicity.

11.6 General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator(s).

12.0 Drug Information

12.1 Docetaxel

12.1.1 Chemistry: Docetaxel is prepared by semisynthesis of the noncytotoxic precursor 10-deacetyl baccatin III that is extracted from the renewable needles of the European yew tree. Structurally, docetaxel is identical to paclitaxel except that modification in two side chains of paclitaxel increase the water solubility of docetaxel.

12.1.2 Biochemistry: Docetaxel binds to free tubulin and promotes its assembly into microtubule bundles while inhibiting their disassembly. This stabilization of microtubules interferes with their normal function and result in inhibition of cell mitosis.

12.1.3 Human pharmacokinetics: Following the intravenous administration of docetaxel its pharmacokinetic profile is consistent with a three compartment model with half-lives for the α, β, and γ phases of 4 minutes, 36 minutes, and 11.1 hours, respectively. The initial rapid decline is due to distribution to peripheral compartments while the late terminal phase is partially due to slow efflux from peripheral compartments. The mean total body clearance is 21 L/hour/m^2 and the mean steady state volume of distribution is 113L. *In vitro* studies showed that docetaxel is about 94% protein bound and is metabolized by the CYT3A4 isoenzyme. It is likely that CYT3A4 inhibitors or inducers such as ketoconazole, erythromycin, troleandomycin, nifedipine, cyclosporine or terfenadine modify docetaxel blood concentration. To date, no formal clinical studies have been performed to evaluation these interactions. The main route of docetaxel elimination is fecal excretion as metabolites. Within 7 days of intravenous administration, 6% and 75% of the dose is excreted via the urine and feces, respectively.

12.1.4 Human toxicology:

12.1.4.1 Hematological: Severe leukopenia and neutropenia that increase susceptibility to infection, febrile neutropenia, anemia and thrombocytopenia. Reversible bone marrow suppression was the major dose limiting toxicity of docetaxel in clinical trials with a median time to nadir of seven days and a median duration of severe neutropenia of seven days.

12.1.4.2 Hypersensitivity reaction: Within few minutes of the start of the infusion patients may develop severe hypersensitivity reaction with hypotension, bronchospasm or generalized rash/erthema. Other events include chills, rigors, back pain, drug fever, dyspnea, and chest tightness. These events are usually resolved after discontinuation of the infusion and administration of appropriate therapy. Premedication with corticosteroids for three days starting one day prior to docetaxel administration reduces the severity of hypersensitivity.

12.1.4.3 Fluid retention: Peripheral edema, general edema and weight gain that are occasionally associated with pleural effusion, dyspnea at rest, pericardial effusion, ascitis or abdominal distention were reported. The incidence and severity are reduced with premedication with corticosteroids.

12.1.4.4 Skin: Severe skin toxicity including localized erythema with edema followed by desquamation has been observed. Rash, including localized eruptions, usually associated with pruritus have been seen. Nail disorders include hypo- or hyperpigmentation and pain. Total alopecia is common.

12.1.4.5 Neurological: Reversible neurological toxicology manifested as parasthesias or dysesthesia, pain and peripheral motor neuropathy.

12.1.4.6 Asthenia: Symptoms of fatigue and weakness may last from few days to few weeks.

12.1.4.7 Hepatic: Increase in bilirubin, transaminases, and alkaline phosphatase were observed. Patients with increased bilirubin and liver enzymes are at increased risk of severe adverse effects.

12.1.4.8 Gastrointestinal: Nausea or vomiting and /or diarrhea are generally mild to moderate.

12.1.4.9 Stomatitis, including severe stomatitis, has been reported.

12.1.4.10 Muscle: Arthralgias and myalgias were reported Local site reaction: Infusion site reactions include hyperpigmentation, inflammation, redness or dryness of the skin, phlebitis, extravasation or swelling of the vein.

12.1.4.11 Pregnancy and lactation: Docetaxel is pregnancy category D. Mothers should discontinue nursing before receiving docetaxel.

12.1.5 Pharmaceutical Data:

12.1.5.1 Formulation: Docetaxel is commercially available as Taxotere®. It is supplied as a clear, viscous, yellow to brown-yellow solution containing 40mg/mL anhydrous docetaxel in polysorbate 80. This pyrogen-free, non-aqueous solution is available in single use vials of 20 mg and 80 mg. Taxotere vials are provided with diluent vials containing sterile, non-pyrogenic 13% Ethanol in Water.

12.1.5.2 Storage and stability: Prior to dilution, Taxotere vials are stored between 2° and 25°C (36° and 77°F) in the original package to protect from bright light.

12.1.5.3 Preparation: Since docetaxel is a cytotoxic drug it should be handled with caution and according to the procedures of proper handling and disposal of anticancer drugs.

12.1.5.4 Preparation of the initial diluted solution:

12.1.5.4.1 The whole content of the appropriate diluent vial is aseptically drawn into a syringe and transferred into the Taxotere for Injection Concentrate. The concentration of the diluted docetaxel is 10mg/mL.

12.1.5.4.2 The diluted solution should be rotated gently for 15 seconds for mixing and allowed to stand for few minutes to let any foam to dissipate. The diluted solution should be clear.

12.1.5.4.3 The initially diluted solution may be used immediately or stored refrigerated or at room temperature for up to 8 hours.

12.1.5.5 Preparation of the final dilution for infusion:

12.1.5.5.1 The required amount of docetaxel 10mg/mL should be aseptically withdrawn with a graduated syringe and injected into an infusion bag (polypropylene or polyolefin, not PVC) or bottle (glass of polypropylene) of 0.9% sodium chloride solution or 5% Dextrose in Water. The final concentration should be between 0.3 and 0.74 mg/mL.

12.1.5.5.2 The solution needs to be mixed by manual rotation and inspected visually for particulate matter or discoloration. If the initial diluted solution or final dilution is not clear or appears to have a precipitation it should be discarded.

12.1.5.5.3 The diluted docetaxel solution is stale between 2° and 25°C (36° and 77°F) for 4 hours and should be used within 4 hours of preparation (including the 1 hour of intravenous administration).

12.1.6 Administration: The final docetaxel diluted for infusion is administered intravenously over 1 hour through a polyethylene–lined tubing (non-PVC).

12.1.7 Supplier: Docetaxel is commercially available and should be purchased by a third party.

12.2 Cisplatin

12.2.1 Biochemistry: cis-diamminedichloroplatinum (cis-DDP) is a heavy metal compound which a divalent platinum molecule binds two potential leaving groups, the chloride ions. Two NH_3 groups are bound in a firm covalent linkage in transposition to the chloride moieties. Both chloride ions undergo a slow displacement by water, generating a positively charged aquated complex, which is capable of interacting with a nucleophilic site on DNA, RNA, or protein.

12.2.2 Pharmacokinetics: The dominant action of cisplatin appears to be inhibition of the incorporation of DNA precursors, although protein and RNA synthesis are also inhibited. Plasma levels of cisplatin decay in a biphasic mode with an initial half-life of 25-49 minutes, and a secondary phase ranging from 58-73 hours. This prolonged phase is due to protein binding, which exceeds 90% of the activity in the second phase. Urinary excretion is incomplete with only 27-45% excreted in the first five days. Largely unchanged drugs are the initial fraction excreted. Although this drug seems to act as an alkylating agent, there are data to indicate that its mode and sites of action are different from those of nitrogen mustard and the standard alkylating agents.

12.2.3 Pharmaceutical Data:

12.2.3.1 Formulation: Cisplatin (Platinol®) is available as an aqueous solution in a concentration of 1 mg/mL.

12.2.3.2 Storage and Stability: The aqueous solution is stored at room temperature protected from light. Cisplatin is stable in mannitol/NS/ D_5W mixtures for 48 to 72 hours at room temperature and up to 72 hours when refrigerated (per Trissel's *Handbook on Injectable Drugs,* 11th edition). Cisplatin should be given immediately after preparation over one hour.

12.2.4 Human Toxicology: Human toxicity from cisplatin includes: nausea, vomiting, anorexia, loss of taste, renal toxicity (with an elevation of BUN, creatinine, and impairment of endogenous creatinine clearance, as well as renal tubular damage, which appears to be transient), ototoxicity (with hearing loss, which initially is in the high-frequency range, as well as tinnitus), peripheral neuropathy, allergic reactions, and uricemia. Much more severe and prolonged toxicity has been observed in patients with abnormal or obstructed urinary excretory tracts. Myelosuppression, often with delayed erythrosuppression, is expected. In the high-dose treatment regimen with osmotic diuresis, the nadir of white blood cells and platelets occurred regularly at about two weeks with recovery generally at about three weeks after the initiation of therapy.

12.2.5 Supplier: Cisplatin is commercially available, and should therefore be purchased by the third party.

12.3 Carboplatin

12.3.1 Chemistry: Carboplatin (CBDCA) is a hydrophilic platinum coordination compound and is an analog of cisplatin, producing intrastrand DNA cross-links.

12.3.2 Human Toxicology: Side effects of CBDCA include: myelosuppression, nausea, vomiting, abdominal pain, diarrhea, and constipation. Other toxicities include: allergic reactions (including hypersensitivity, i.e. rash, urticaria, erythema, pruritis, bronchospasm, and profound hypotension), peripheral neuropathy, paresthesias, loss of hair, hearing loss, visual disturbances, and change in taste. Serum creatinine elevations and blood urea elevations have occurred as well as abnormal liver function tests and decreased serum electrolyte values. Although rare, pain asthenia, cardiovascular, respiratory, genitourinary, and mucosal side effects have occurred in some patients. Cancer-associated hemolytic uremic syndrome has been reported rarely carboplatin may cause fetal harm; therefore, women of childbearing potential should be advised to avoid becoming pregnant. The renal effects of nephrotoxic compounds may be potentiated by carboplatin. Carboplatin is contraindicated in patients with a history of severe allergic reactions to cisplatin or to other platinum-containing compounds or mannitol. This drug should not be used in patients with severe bone marrow depression or significant bleeding. The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

12.3.3 Pharmaceutical Data

12.3.3.1 Kinetics: The differences in potencies of carboplatin as cisplatin are due to differences in equation rates. The initial half-life of carboplatin is 1.1-2.0 hours and the post-distributional half-life is 2.5-5.0 hours. Sixty-five percent of the dose is excreted into the urine within twelve hours. Carboplatin is not bound to plasma proteins.

12.3.3.2 Formulation: Carboplatin is supplied as a sterile lyophilized powder available in single-dose vials containing 50 mg, 150 mg, and 450 mg of carboplatin for administration by intravenous injection. Each vial contains equal parts by weight of carboplatin and mannitol. Immediately before use, the content of each vial must be reconstituted with either sterile water for injection, USP, D_5W, or normal saline injection, USP, according to the following schedule:

Vial Strength	Diluent Volume
50 mg	5 mL
150 mg	15 mL
450 mg	45 mL

These dilutions all produce a carboplatin concentration of 10 mg/mL. Carboplatin can be further diluted to concentrations as low as 0.5 mg/mL with D_5W or normal saline injection, USP.

12.3.3.3 Storage and Stability: Unopened vials of carboplatin for injection are stable for the life indicated on the package when stored at controlled room temperature (15-30°C), and protected from light. When reconstituted as directed, the solution of carboplatin exhibits no decomposition for eight hours at room temperature (25 °C). Like cisplatin, this drug should not be given through aluminum needles. **Caution:** The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded eight hours after dilution.

12.3.3.4 Administration: Intravenous infusion.

12.3.4 Supplier: Carboplatin is commercially available and should be purchased by a third party.

12.4 AT-101

12.4.1 Description: AT-101 is the levorotatory enantiomer of gossypol acetic acid, R-(-)-gossypol acetic acid; the chemical name is R-(-)-1,1', 6,6',7,7'-hexahydroxy-3,3'-dimethyl-5,5'-bis (1-methylethyl) [2,2'-Binapthalene]-8, 8'-dicarboxaldehyde acetic acid. AT-101 will be administered orally using an immediate release (IR) solid oral dosage form. Each AT-101 tablet contains 10 mg R-(-)-gossypol acetic acid. In addition, each tablet of AT-101 also contains excipients.

12.4.2 Toxicity Associated with AT-101:

As of 23 June 2009, approximately 699 patients have received AT-101 across multiple Ascenta-sponsored Phase I and Phase II trials as a single agent or in combination with other anticancer therapies. An additional 92 patients have been treated under a separate IND by the DCTD of CTEP. The most frequent adverse events associated with AT-101 dosing have been gastrointestinal findings such as nausea and vomiting; anorexia and fatigue have also been frequent. Most adverse events have been of low grade (Grade 1-2 on the NCI Common Toxicity Criteria scale), but a clinical picture of ileus/non-mechanical small bowel obstruction has also been observed in approximately 10-15% of patients receiving 30 mg of AT-101 daily or daily for 21 of 28 days. Following these initial observations, as part of Ascentage's ongoing pharmacovigilance program, Ascentage has modified the dosing schedule of single-agent AT-101 and reduced the dose when administered daily for multiple cycles (from 30 mg/day to 20 mg/day for 21/28 days). The incidence of ileus/non-mechanical small bowel obstruction was reduced to approximately 5% in patients treated with reduced doses of AT-101 daily for 21 of 28 days.

Ileus/non mechanical small bowel obstruction has also been observed as a DLT in patients treated with alternative schedules of AT-101. The MTD for b.i.d. x 3 day administration of AT-101 every fourteen (14) days has been determined to be 30 mg b.i.d. and for weekly administration is 80 mg. The incidence of the most common Grade 1-2 and grade 3-4 AEs (nausea, vomiting, fatigue, and diarrhea) were lower using these alternative schedules of AT-101. Among approximately two hundred fourty (240) patients treated with AT-101 40 mg b.i.d. days 1-3 in combination with docetaxel 75 mg/m2 day 1, q 21 days, there have been 2 reports of drug-related ileus/non-mechanical small bowel obstruction, including one in a patient treated on a randomized blinded trial. In a completed, blinded, randomized trial in 2L NSCLC in one hundred five (105) patients testing the combination of docetaxel (75 mg/m2 day 1, q 21 days) and AT-101 (40 mg b.i.d. days 1-3, q 21 days) compared to docetaxel and placebo, unblinded AE tables and listings have been reviewed. There were no notable differences in AE incidence or severity for the placebo treated patients compared to the AT-101 treated patients. This supports the conclusion that severe gastrointestinal toxicity is less common when AT-101 is dosed intermittently and that AT-101 is well tolerated in combination with docetaxel using these doses and schedules of administration.

The AT-101 pharmacovigilance program included monitoring of troponin and electrocardiograms. AT-101 administration may be associated with a risk of developing troponin elevations; however, these elevations were generally minimal, asymptomatic and not associated with drug-related SAEs of the cardiovascular system. The importance of identifying these elevations is uncertain at this time. In the completed, blinded, randomized trial of AT-101 or placebo in combination with docetaxel in 2L NSCLC (CS-204, N=105), unblinded AEs have been reviewed. There were similar numbers of patients who were reported to have elevated troponin values who received AT-101 compared to placebo. Troponin monitoring is no longer recommended as part of routine pharmacovigilance.

12.4.3 Medications Prohibited From Use with AT-101:

Patients may not receive any of the following medications or therapies while receiving study treatment:

- Other investigational agents are not to be used within 4 weeks of initial dosing or while receiving treatment.
- Concurrent approved or investigational anti-cancer therapy (e.g., chemotherapy, immunotherapy, targeted therapy, biologic therapy) other than protocol therapies.

12.4.4 Study Drug Packaging and Labeling**:**

AT-101 10 mg tablets will be packaged in high density polyethylene (HDPE) opaque bottles, each containing 24 tablets. The bottles will employ a child-resistant polypropylene cap with pressure sealed liners. Each bottle will be labeled in the appropriate language, including the required regulatory text for each country and labeled with a bottle number.

12.4.5 Study Drug Storage:

AT-101 tablets should be stored under refrigeration, (2°C-8°C) and protected from direct heat. To assure maximal stability, AT-101 should not be handled at ambient temperatures for more than 24 hours (e.g. for the purpose of delivery or dispensing). During shipments, temperature deviations of up to 25°C for 24 hours and greater than or equal to -20°C for 16 hours fall within current stability conditions and hence would allow for continued use of clinical trial material. Ascentage will assure that the stability of the drug is monitored to ensure suitability for continued use in the clinic.

13.0 Radiation Therapy

13.1. General Considerations: All the patients in this study will receive either definitive radiotherapy or post-operative irradiation. Definitive radiotherapy will begin within three weeks following the induction cycle of chemotherapy. Post-operative radiation should begin as soon as adequate healing has been established. Usually, this will be within three to four weeks of the surgical procedure but must begin by six weeks.

13.2 Radiation Fields: The treatment volumes will be individual as for each patient depending upon the extent of disease. Tumor volumes will be outlined on the planning CT scans with the aid of pre-chemotherapy CT and, if available, PET scans, to ensure adequate irradiation of the pre-chemotherapy tumor volume. Treatment techniques will aim at adequate irradiation of the clinical and the sub-clinical disease. The therapy goals, specifying the intended doses to the primary tumor and lymph node metastases, and the intended doses to each lymph node level treated adjuvantly, will be detailed in the therapy chart. A CT-based display of the isodoses will be recorded, such that it will be feasible to assess whether the intended (prescribed) isodoses cover the targets adequately.

13.3 Doses:

13.3.1 Radiation with chemotherapy: Tumor doses will be expressed in Gy . The prescribed doses should encompass the targets. Treatment plans will be generated demonstrating adequate coverage of the target volume. The dose across the target volume should not vary by more than +/- 10% of the prescribed dose. When an anterior-posterior low neck field is treated, the dose will be prescribed to 3cm depth. For electron beam treatments, the dose will be prescribed to the depth at which maximum dose is obtained (Dmax). When treating the posterior cervical nodes, either six or nine MeV electrons may be used. Treatment will be given once daily, five days per week, two Gy per fraction to gros disease, and 1.6-2.0 Gy per fraction to subclinical disease. Total gross dose will be 70 Gy and subclinical disease dose will be 50-60 Gy.

13.3.2 Post-Operative Radiation Therapy: The patient will be treated with conventional fractionation, 1.8-2.0 Gy per fraction, five fractions per week in a continuous course. The dose to the tumor bed and lymph nodes will be 56-64 Gy, depending of the existence of extranodal extension or the existence of close surgical margins. Patients with gross residual disease or positive resection margins will receive total 66-70 Gy to the sites of residual disease.

13.4 Immobilization and Positioning: All patients will be treated in a position that affords maximal daily reproducibility. Commonly, it will be in the supine position. Immobilization devices such a head masks or bite blocks are mandatory to ensure that target volumes are adequately treated.

Cradles or arm restraints may be needed to allow adequate exposure of the lower neck area in selected patients.

13.5 Simulation and CT Scanning: All patient will undergo simulation including a CT scanning. Treatment planning CT scans with contrast will be obtained on each patient prior to or during the first week of the first cycle of chemotherapy to ensure adequate radiation of the pre-chemotherapy tumor extent.

13.6 Technical Factors: Equipment - Megavoltage equipment with a source to skin distance of 80-100cm (or source-axis distance), or greater, will be used. Megavoltage machines with an energy equal to 6 MeV photons will be used, rarely higher energy may be used if necessary.

13.7 Treatment Planning: CT based planning may be used for the total course of radiation, especially if necessary for parotid sparing purposes. Otherwise, the final boost may be planned or the full course of radiation may be planned using orthogonal simulation fields alone, using the information from the pre-chemotherapy planning CT for verification of the adequacy of the radiation fields.

13.8 Treatment Interruptions: It is expected that the entire treatment for definitive irradiation will be completed in about seven weeks. Treatment interruptions due to symptomatic mucositis or skin reactions should be minimal. In the case of severe mucositis, a gastric tube will be inserted and radiation will continue uninterrupted at the discretion of the treating physician. Weight will be recorded weekly in the Radiation Oncology chart. If the patient's weight loss exceeds 10% of the initial weight or if the patient is malnourished before radiation, a feeding tube will be inserted.

14.0 Salvage Surgery

14.1 The extent of salvage surgery for either the primary tumor or regional nodes is dictated by the extent of tumor present at time of initial tumor staging or may be increased if tumor progression has occurred.

14.2 Salvage surgery may be required at the times of scheduled tumor assessments or any time tumor progression or recurrence is demonstrated by adequate biopsies of areas clinically suspicious for tumor involvement.

14.3 Primary site: total laryngectomy will be required in all cases except those rare situations in which a partial laryngectomy can be safely and adequately performed based on careful pre-treatment and post-treatment assessment of tumor extent (see 14.1).

14.3.1 Neck: Bilateral selective neck dissections will be performed in conjunction with salvage total laryngectomy for any patient initially staged N_0 in the neck. Ipsilateral modified radical or radical neck dissection is required for any patient initially staged N+ who recurs or persists with cancer in that neck. Neck dissection alone without laryngectomy is required for any patient who is PET positive or with biopsy proven palpable neck diseaseat the discretion of the treating surgeon. Neck dissection alone is also required any time during the follow-up period that the neck recurrence is documented and satisfactory negative biopsies are obtained from the primary tumor site.

14.4 Primary closure of the surgical defect is to be accomplished whenever possible. Reconstructive closure with grafts or free flaps is at the discretion of the surgeon.

15.0 Informed Consent

15.1 Alternative treatment options include total laryngectomy followed by radiation therapy with or without chemotherapy or radiation therapy alone.

15.2 Patients who meet the inclusion criteria will be approached for possible participation in this study. The nature of the investigation will be described to the patient including the risks and side effects of study treatments, the potential benefit of the study to themselves and others, and the time commitment and frequency of patient visits and the clinical evaluations they will be required to undergo. The patient will then have the opportunity to ask questions. The patient will be given the appropriate informed consent form for consideration. Each patient will be allowed to read (or have read to them) the informed consent form and understand before discussing consent with the investigator. If consent to participate is granted, the patient's signature will be obtained on the informed consent form. The original consent form will be kept in the patient's chart at the Clinical Trials Office. Copies of the informed consent will be provided to the patient, and will also be available to the principal investigator and the nurse coordinator.

16.0 Patient Registration

16.1 All patients will be registered with the University of Michigan Comprehensive Cancer Center Clinical Trials Office prior to initiating treatment.

17.0 Reporting Potentially Serious Adverse Events

17.1 Definitions

17.1.1 **An adverse event** is any new, undesirable medical experience or change of an existing condition which occurs during or after treatment, whether or not considered product-related.

17.1.2 **A serious adverse event** is any untoward medical occurrence that suggests significant hazard or side effect that:

1. results in death.
2. is life-threatening (places the patient at immediate risk of death).
3. requires or prolongs inpatient hospitalization
   is disabling or incapacitating.
4. is a congenital anomaly/birth defect.

The definition of serious adverse event (experience) also includes important medical event. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive

treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

17.1.3 Attribution: Definitions of relationship to study medication are as follows:

UNRELATED: The AE is clearly ***NOT*** related to the intervention/investigational agent.

UNLIKELY: The AE *is* ***doubtfully related*** to the intervention/investigational agent.

POSSIBLY: The AE ***may be related*** to the intervention/investigational agent.

PROBABLY: The AE ***is likely related*** to the intervention/investigational agent.

DEFINITELY: The AE ***is clearly related*** to the intervention/investigational agent.

Additionally, adverse events should be categorized as ***expected or unexpected***. Adverse events not attributed to the chemotherapy agents will be considered unexpected. Adverse events attributable to the chemotherapy will be reported if the adverse events are at an intensity that is more severe that previously documented or considered significant by the investigator. The definition of "related" is that there is a reasonable possibility that the drug caused the adverse experience.

17.2 Reporting Procedures

"Serious" adverse events which occur after the initial dose of AT-101, during treatment, or within 30 days of the last dose of AT-101 (including all deaths), MUST be reported immediately, i.e., within one day (24 hours) of being identified, to the study coordinator at the Clinical Trials Office, and to the Ascentage Pharma Group Corporation (APGC) Pharmacovigilance (PV) Department to drugsafety@ascentagepharma.com.

An initial written report of the serious adverse event must be prepared by the investigator using the MedWatch Form (Form FDA 3500 A), and emailed within 48 hours. This report should provide a detailed description of the adverse event and include copies of hospital records and other relevant documents, when possible. Autopsy results, if applicable, should also be sent as soon as they become available. Copies of each report will be kept in the Investigator's File and patient's research chart. The investigator will submit, on request, copies of all these reports to the relevant ethics committee. Follow-up will be conducted by Ascentage Pharma Group Corporation (APGC) as needed with investigational site personnel to obtain any additional information needed to complete the reporting of the event.

All fatal or life threatening serious adverse events must be reported to the Ascentage Pharma Group Corporation (APGC) Medical Monitor IMMEDIATELY and the initial SAE report must be emailed to Ascentage Pharma Group Corporation (APGC), as described previously, within 24 hours.

All adverse events will be noted in the study forms. Any serious adverse events, unexpected adverse events or adverse events $\geq$ grade 3 will be reported immediately to the principal investigator. All serious adverse events will be reported on Form FDA 3500 A (MedWatch Form)

17.2.1 Reporting to the Medical IRB:

The investigator or delegate is responsible for notifying the IRB/IEC of serious adverse events in accordance with the policy of the IRB/ IEC.

17.2.2 Serious Adverse Events Reporting to the FDA:
The Clinical Trials Office Staff will coordinate with the Michigan Institute for Clinical and Health Research (MICHR) IND/IDE Investigator Assistance Program (MIAP) office for the reporting of any and all IND safety reports to the FDA as per the requirements outlined in 21 CFR 312.32.

A summary of all non-expedited safety reports will be submitted in the annual report.

18.0 Data and Safety Monitoring

Scheduled meetings will take place **monthly**. Attendees at these meetings will include the protocol investigators (will always include the PI), the CTO data manager, the CTO regulatory manager, the Cancer Center clinical research nurse, the biostatistician, and the clinic nurses, all of whom are involved with the conduct of the protocol. The University of Michigan Cancer Center DSMB will oversee this clinical study.

During these meetings the investigators will discuss matters related to:

1. safety of protocol participants (AE reporting)
2. validity and integrity of the data
3. enrollment rate relative to expectation, characteristics of participants
4. retention of participants, adherence to protocol (potential or real protocol violations)
5. data completeness

Data and Safety Monitoring Reports of these regular meetings will be kept on file in the Cancer Center Clinical Trials Office. The data manager assigned to the trial will be responsible for completing the report. The reports will be signed by the principle investigator or by one of the Co-PI.

The University of Michigan Comprehensive Cancer Center's Data Safety Monitoring Board will meet on a monthly basis to review the prior months SAEs and Data and Safety Monitoring study specific reports that have been filed.

19.0 Biologic Correlatives

19.1 Biomarker Anaylsis

Overexpression of p53, p16 $^{(INK4a),}$ EGFR, VEGF, and apoptosis inhibitors of the Bcl_2 family are consistently identified markers of increased risk for head and neck cancer recurrence. Inactivation of p16 maybe a central defect by allowing overexpression of COX-2 in response to smoking and other stress signals and thereby result in secondary increases and activation of MDM2, p53, nuclear p65 and increased serum levels of IL6 and VEGF. The role of p16 in laryngeal cancer is still unknown. However, we have previously demonstrated that both p53 mutation status and low Bcl-xL expression are significant predictive factors for response to induction chemotherapy and larynx preservation in two prospective trials. We hypothesize that AT-101 can modulate the expression of Bcl-xL, and Bcl-2, in tumor and adjacent non-tumor mucosa in patients with head and neck cancer. To understand the effects of AT101, we will need to know the status of p53 expression. We will also explore other potentially important markers such as p16, VEGF and EGFR expression. Loss of p16 has been identified as a

critical event in breast cancer carcinogenesis and leads to increased COX-2 expression in response to oxidative stress factors such as smoking or chronic inflammation. Most patients with laryngeal cancer are chronic smokers. Hypermethylation of p16 is frequent in HNSCC tumor tissue, adjacent mucosa and saliva. The p16 locus also encodes p14 ARF which normally activates p53 and inhibits NF-kB. In cancers without p14/p16, a defect in p53 activation occurs and NF-kB activation can promote Bcl-xL expression and survival rather than repress anti-apoptotic genes. If these observations are related, p16 defective tumors should have increased NF-kB, phospho p65 and/or Bcl-xL expression. We will collect pre and post treatment tumor and serum samples to measure these biomarkers. These samples will be collected at the baseline visit and and after one cycle of docetaxel/platinum induction chemotherapy and at time of any salvage surgery and are for research purposes. They will be processed for the appropriate assays and formalin fixed histopathologic blocks will be obtained from the Pathology Department for creation of a tissue microarray (TMA) that will be utilized for immunhistologic analysis. DNA will be prepared from each sample at time of TMA creation. Each patient and corresponding samples will be assigned a unique SPORE (BioDBx) number and will be stored for analysis in the Tissue Core Laboratory at the University of Michigan (Room 5110 Cancer Center). These research studies will be paid for by the research study. These collections and processing are routine procedures for our SPORE study participants.

19.2 CT Perfusion analysis

CT perfusion will be performed to establish baseline values for tumor angiogenesis. CT perfusion scans will be repeated after the first cycle of therapy to evaluate tumor vascular perfusion in response to treatment. CT perfusion studies have shown that tumors that demonstrate increased blood flow and blood volume frequently respond better to induction chemotherapy and chemoradiation compared with tumors that have decreased blood flow and blood volume. Our preliminary studies have also shown correlation of blood flow and blood volume in tumors will correlate positively with p16 status, and negatively with EGFR, suggesting that CT perfusion can be a noninvasive predictor of these important biomarkers [34]. Other biomarkers have shown correlations with CT perfusion as it relates to angiogenesis. In particular, other published data from the University of Michigan demonstrates that the serum biomarker CXCL8 is significantly correlated with CT capillary blood flow, which is likely secondary to increased angiogenesis in locally advanced head and neck tumors [42].

20.0 Statistical Considerations

Design

This is a Phase II, single-arm study with an initial randomized component. The primary clinical objective of this trial is to compare the larynx preservation rates in a treatment paradigm that uses induction chemotherapy plus AT-101 to select patients for either concurrent chemoradiation or surgery. Organ preservation rate, defined as alive and free from indication for laryngectomy three months post treatment, was chosen as the primary endpoint because it provides evidence to fully characterize clinically the effect of the treatment strategy

Patients will be randomly assigned to one of two treatment arms and receive arm specific therapy for one cycle. After 1 cycle, subjects who have complete response will undergo chemoradiation. Patients without complete response will receive a second neoadjuvant course of TP plus AT-101 for their second cycle and be reassessed for response. After completion of the second cycle, those with response (CR+PR) will undergo chemoradiation while those with NR will undergo surgery.

We incorporate an early stopping rule into the design for overall salvage laryngectomy rates at 3 months. The treatment paradigm will be considered promising if the overall salvage laryngectomy rate is 30% or less. Alternatively, if the rate of salvage laryngectomy is over 50%, there would be little interest in pursuing the therapy in further studies. In this case, we would like to stop accrual to the trial and call a meeting to discuss discontinuation. The optimal two-stage accrual design [Simon, Controlled Clinical Trials 10:1-10 1989] has been adopted.

In the first stage, we will accrue 17 response-evaluable patients. In order for a patient to be evaluable, two response evaluations must be available. Patients who do not undergo two response evaluations will be replaced.If 8 or more patients have had a laryngectomy anytime up to 3 months after completion of treatment of the 17th patient, the trial will stop accruing and a meeting will be called to discuss discontinuation. If 10 or more patients have achieved early larynx preservation we will continue and accrue an additional 35 patients. Based on all 52 subjects, if 31 or more patients have achieved successful organ preservation, further studies will be proposed.

Randomization

The purpose of the randomization is to enable a comparison between TP and TP+AT101 based on the clinical and laboratory measures that will be assessed after one cycle.

Randomization, TP+AT-101:TP alone will be 2:1, stratified by clinical stage (III or IV) and blocked to ensure balance in treatment assignments. The study statistician will set up the randomization prior to beginning the study.

Sample Size and Design Justification

This trial design provides 85% statistical power and 5% overall type I error rate for detecting early larynx preservation rates of 50% versus 70%. Table 1 gives the operating characteristics of this design under various scenarios for the underlying population response. For example, if the therapy has a larynx preservation rate of only 40%, there would be a 91% chance of stopping the trial after just 17 patients. If the therapy leads to organ preservation (salvage laryngectomy) in 70% (30%) of patients, only 11% of trials using this design would terminate early after enrolling 17 subjects. Furthermore, because this study has 85% power, 85% (15%) of trials using this design would conclude that the therapy is effective (ineffective). The expected sample size is 48.2 subjects because 89% of trials would accrue all 52 subjects, while 11% of trials would accrue only 17 subjects.

Table 1: Design operation characteristics for Larynx preservation rates

	Larynx preservation rate (Salvage Laryngectomy rate)						
	40% (60%)	50% (50%)	55% (45%)	60% (40%)	65% (35%)	70% (30%)	75% (25%)
Probability of terminating the trial at first stage	0.91	0.69	0.53	0.36	0.21	0.11	0.04
Overall probability of rejecting the therapy	1.0	0.95	0.83	0.61	0.34	0.15	0.04
Expected sample size	20.15	27.9	33.5	39.4	44.7	48.2	50.6

Note: Computation is based on 17 evaluable patients at first stage and 52 total evaluable patients at the end of the trial, with the constraint of stopping the trial if 9 or less subjects with preserved larynx at the first stage or if a total of 31 or fewer subjects have preserved larynx at the end of second stage.

If total accrual is met, the trial will provide an estimate of overall clinical (CR or PR) response after one cycle of TP+AT101 from approximately 35 patients. The operating characteristics for response rate estimates ($\hat{\boldsymbol{p}}$) with n=35 are summarized in Table 2 under various scenarios of true response (**p**). Table 2 is based on 10,000 simulations and binomial exact confidence intervals. For example if the true response rate is 75%, 47% of trials would observe a rate greater than 0.75, while the lower bound of a 95% confidence interval will only be greater than 0.75 in 2% of trials.

True p	Proportion of Trials where ($\hat{\boldsymbol{p}}$) > 0.75	Proportion of Trials where 95% CI lower bound of ($\hat{\boldsymbol{p}}$) >0.75
0.75	0.47	0.02
0.80	0.75	0.06
0.85	0.93	0.21
0.90	0.99	0.53
0.95	1.00	0.91

The University of Michigan will accrue patients to the study. Based on previous experience, the accrual to this trial is expected to be 15 eligible patients per year. Thus, it will take approximately 14 months to reach 17 response-evaluable patients for the first stage, and the accrual period required to reach the overall target of 52 response-evaluable patients is expected to be approximately 3.5 years.

Analysis Plan

Overall salvage laryngectomy rate will be assessed 3 months after completion of surgery or chemoradiation. Successful organ preservation will be defined as a patient who is alive and free of indication for surgery. Patients with no response to induction chemotherapy but do not undergo surgery will be considered in the indication for surgery group. The rate of successful organ preservation will be compared to the same measure in UMCC9520.

Progression-free survival will be defined as the time from randomization to the time of first indication of local failure or metastases.

Overall response rate (CR +PR) after first cycle of chemotherapy will estimated for the TP+AT101 group and compared to the TP alone arm as well as the rate observed after 1 induction chemotherapy cycle in UMCC 9520.

We plan to look at the data from all patients for an overall summary of success of the treatment paradigm. We will analyze the two arms of the trial both separately and together. We acknowledge there may be some patients who achieve CR after one cycle of TP alone and never receive AT101. We anticipate that this will be a small proportion (CR was observed in approximately 15% after 1 cycle in UMCC 9520). Not including these subjects in our overall assessment may introduce bias to our conclusions. To avoid this bias, we plan include these patients in the analysis of the treatment strategy under the assumption that they would have responded to TP+AT101 if that was assigned.

Statistical methods

Primary Objectives:

We will perform a two-sided Pearson chi-square test of proportions to test organ preservation rate. We will construct a binomial confidence interval for the estimate of response rate using the exact method. Progression-free survival at 3 years will be estimated non-parametrically using the Kaplan-Meier

method and compared to the rates observed in UMCC 9520. A Cox proportional hazards model will be used to evaluate progression-free survival across the studies adjusting for site (glottis vs. supraglottic) and tumor stage (T3 vs. T4).

Secondary Objectives:

A single Tissue microarray will be created for pre and post treatment biopsy specimens. Bcl2/BclxL, EGFR and p53 expression will be rated by a pathologist blinded to patient data. A mixed model approach will be used to assess if there are significant changes in the mean levels of expression between pre and post treatment specimens. Empiric estimates of the standard errors will be used to avoid bias due to within-subject variability.

21. 0 Quality of Life Analyses

Quality of life (QOL) assessments will be made prior to treatment (i.e. pre-induction chemotherapy) and at 6 months, 12 months, and 24 months (+/- 4 months) after completion of chemo-RT or surgery-RT (or surgery–chemoRT, as indicated). Three QOL instruments will be administered: the University of Michigan Head and Neck Quality of Life Instrument (HN-QOL) [40], the University of Michigan Voice Related Quality of Life Measure (V-RQOL) [39], and the FACT Head and Neck (version 4) (FACT H&N) [41].

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23.0 Appendices

Appendix A: ECOG Performance Status Scale

DESCRIPTION	SCALE
Normal Activity	0
Symptoms of disease, able to carry out activities of daily living	1
Out of bed >50% of time; occasionally needs help	2
In bed >50% of time; needs nursing care	3
Bedridden; may need hospitalization	4

Appendix B

Functional Capacity

Objective Assessment

Class I. Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.

A. No objective evidence of cardiovascular disease.

Class II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.

B. Objective evidence of minimal cardiovascular disease.

Class III. Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.

C. Objective evidence of moderately severe cardiovascular disease.

Class IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

D. Objective evidence of severe cardiovascular disease.

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Appendix B: Univeristy of Michigan HN-QOL (attached)

Appendix C: FACT-HN (version 4) (attached)

Appendix D : University of Michigan V-RQOL (attached)

Appendix E: University of Michigan AT-101 Dosing Diary