TITLE: A Phase 1B/2 Study of *TALADEGIB in Combination with Weekly Paclitaxel, Carboplatin, and Radiation in Localized Adenocarcinoma of the Esophagus or Gastroesophageal Junction

Study Site:	The University of Texas MD Anderson Cancer Center
Protocol Number	2014-0966
Version Date:	November 18, 2015
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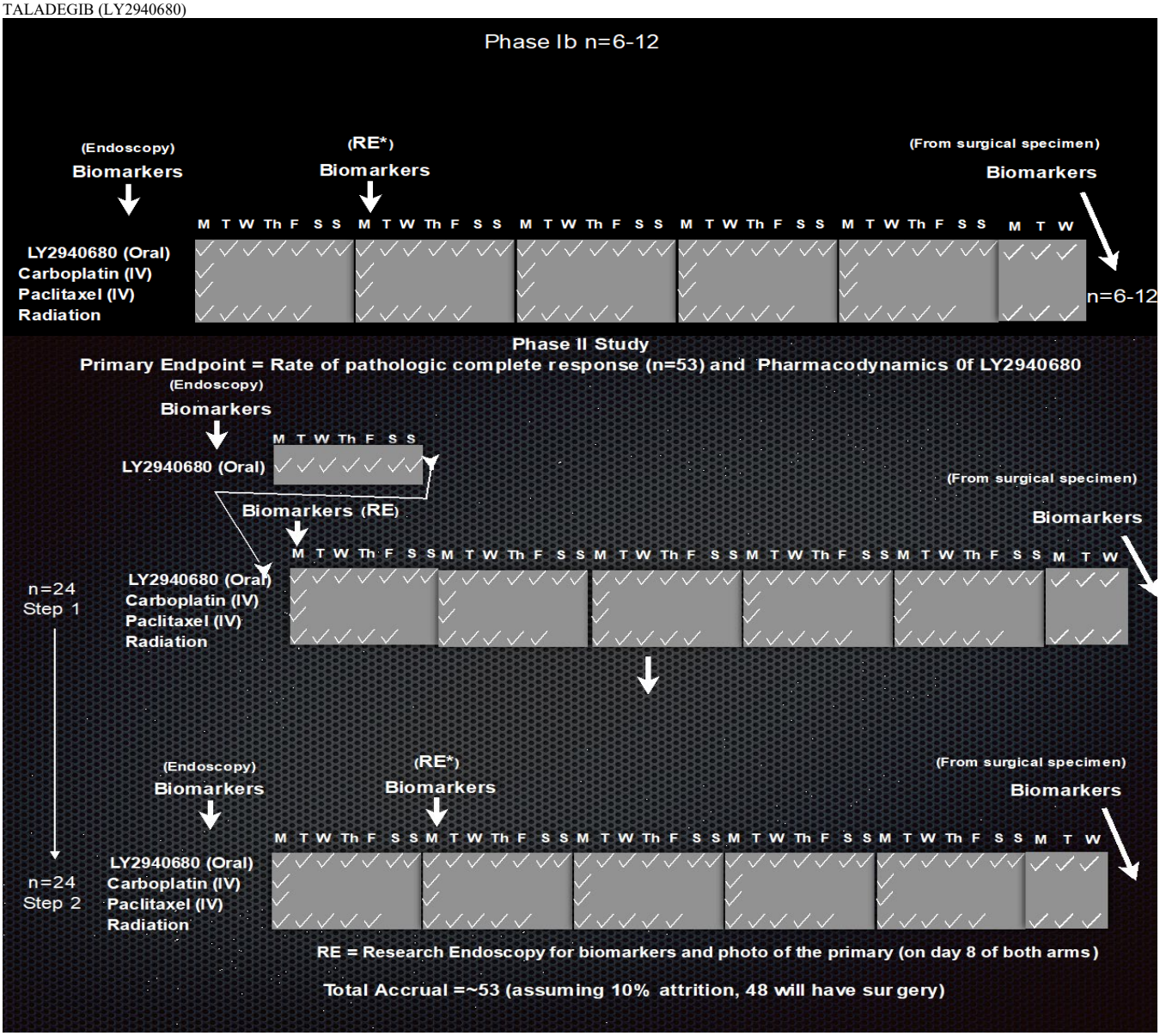
*TALADEGIB (RXDX-109) formally LY2940680

SCHEMA

The general study design is sequential and it is a single-arm study. Please note that the number of patients in phase II will increase to 54 since we expect that 10% of patients will not go to surgery.

TALADEGIB (LY2940680)



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1. OBJECTIVES

1.1 Primary Objectives

1.11. To evaluate the toxicity of TALADEGIB administered orally daily concurrently with weekly paclitaxel, carboplatin and radiation therapy in patients with localized nuclear Gli-1 expressing adenocarcinoma of the esophagus or gastroesophageal junction (Phase IB).

1.12. To assess the rate of pathologic complete response (pathCR) when TALADEGIB is administered orally daily concurrently with weekly paclitaxel, carboplatin, and radiation therapy in patients with localized nuclear Gli-1 expressing adenocarcinoma of the esophagus or gastroesophageal junction (Phase II).

1.2 Secondary Objectives

1.21. To evaluate the toxicity of biochemoradiation in the phase II study.

1.22. To assess additional biomarkers (Hedgehog [Hh] related and Hh unrelated) in sequentially procured tissues (biopsies and resected specimens). *Expression studies:* (1) We will conduct a pre- and post-treatment read-out of the pathway in adenocarcinoma of the esophagus or gastroesophageal junction (EAC) biopsies by determining the expression of Shh, Ih, Gli1 and Gli2, Patch 2 and sFRP1 mRNA using real-time qPCR. (2) We will use the gene expression data to refine and expand the pathway-associated markers listed in item #1 above and attempt to predict sensitivity or/and resistance to therapy in pre-treatment EAC biopsies using real-time qPCR. (3) We will examine the levels and spatial localization of Shh, Ih and Gli-1 using quantitative immunohistochemistry (IHC) in pre- and post-treatment cancer biopsies. (4) We will use fluorescent TUNEL and laser scanning cytometry (LSC) to measure levels of apoptosis in pre- and post-treatment biopsies as a measure of biological response. Biological response will be correlated with measures of Hh pathway inhibition and clinical response.

1.23 Assess if TALADEGIB down modulates its target (Gli-1) in the first cohort (where TALADEGIB will be administered alone for the first 7 days) of the phase II study.

1.2.4 Assess relapse-free survival and overall survival.

2. BACKGROUND

2.1 Adenocarcinoma of the Esophagus

Carcinoma of the esophagus is a virulent disease and there are >600,000 new cases each year.[1] The incidence of EAC has increased rapidly in the West over the past 28+ years.[2-4]. White men more often get EAC compared to white women and other ethnicities.[2, 3, 5] Despite this marked increase in the incidence of EAC, an early diagnosis is not being made and its mortality continues to rise.[3] Localized EAC is diagnosed in ~50% of patients and they are eligible for multimodality therapy including preoperative chemoradiation (CTRT).

The 5-year cure rate from surgery alone is consistently low (≤20%) for the common clinical stage (II or III).[3, 6] Preoperative chemoradiation is now the best strategy for localized EAC, [7-12] however, the outcomes still remain suboptimal. [7-12] One of the major challenges in the treatment of patients with localized EAC is the lack of our ability to optimize therapy that is associated with significant morbidity and mortality.[3, 13, 14] Currently, no biomarker, baseline clinical parameter, or treatment parameter can help one to select a specific treatment or avoid a specific one.[15-17] There is an urgent need to develop an individualized approach to the treatment of localized EAC. Studies of the clinical biology of EAC suggest that there is considerable heterogeneity in the outcome of patients with EAC who seem to have a similar clinical baseline stage and are treated similarly.[6, 14, 18-23] This unpredictable heterogeneity is manifested not only if surgery is used as primary therapy but also when preoperative chemoradiation is utilized.[15, 17, 24-26] After preoperative chemoradiation, the degree of residual cancer (reflection of sensitivity or resistance to chemoradiation) correlates with overall survival (OS) and disease-free survival (DFS). [15, 17, 24-26] Only 20-25% of EAC patients achieve a pathologic complete response (pathCR) and have a 5-year survival (OS) rate of </= 65% but most patients do poorly and patients with considerable amount of cancer (>50% residual cancer[15, 27]; extreme chemoradiation resistance (exCRTR) have the worst outcome.[15, 24, 27, 28] Chemoradiation is quite toxic (nausea, dehydration, fatigue, and weight loss) and surgery is life altering (severe reflux, dumping syndrome, micro-aspirations, and weight loss). Finally, there is agony of brain/appetite and stomach/reservoir disconnect that every patient experiences. Overcoming chemoradiation resistance in patients with EAC could provide a significant advantage. A biomarker-based approach would be rational and will allow optimization of therapy of individual patients. In addition, molecular biology studies have uncovered novel and potentially exploitable targets. Hedgehog (Hh) signaling is commonly observed in EAC. Our preliminary data below suggest that chemotherapy and radiation resistance is likely mediated by activation of the Hh pathway (at least in some cases).

Preliminary data generated at the University of Texas M. D. Anderson Cancer Center

Transcriptional Profiling Identifies Molecular (Hh) Pathways Associated with Resistance to Chemoradiation in EAC. We profiled untreated cancer tissue from patients with esophageal cancer (all had trimodality therapy) using the Affymetrix U133A platform and correlated their molecular signatures with pathologic response. [29] Unsupervised hierarchical cluster analysis segregated cancers into two molecular subtypes. All but one pathCR clustered in subtype I. Thus one subtype appeared less likely to achieve pathCR compared to the other. ~450 genes were differentially expressed between the two subtypes with an estimated false discovery rate of 5%. >2 fold differences in the expression levels were seen for 80 genes using the t-test ($p<0.0001$). Several genes, associated with apoptosis, calcium homeostasis, and stress response were collectively down- regulated in subtype II. Quantitative RT-PCR of several randomly selected genes confirmed the differential expression observed in the microarray data. *Shh* was prominent among the genes with high expression in resistant cancers (Figure 1).

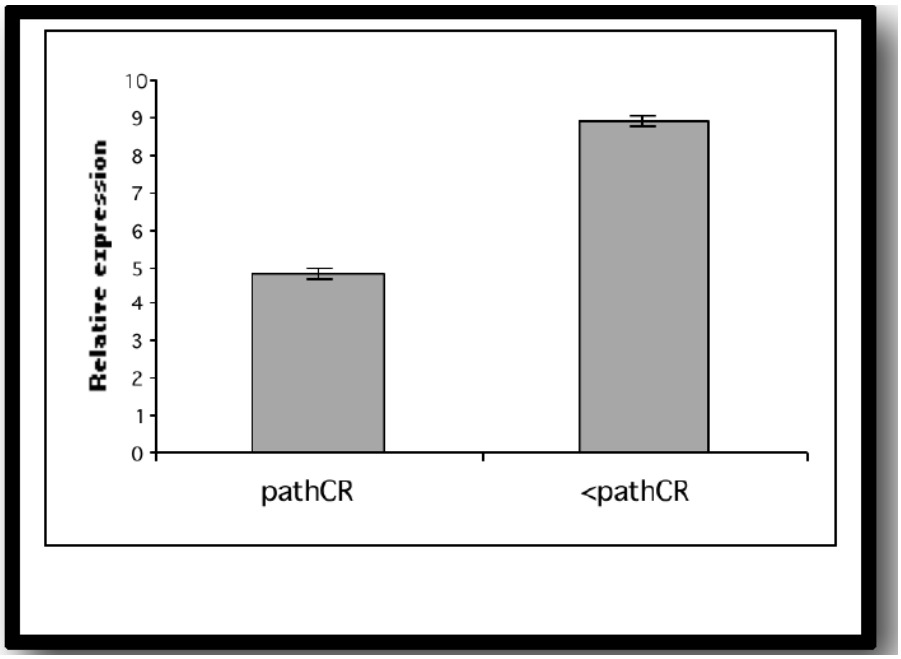


Figure 1. Differential expression of *Shh* by qPCR correlates with response to chemoradiation

Limited RNA (in small number) was studied for *Shh*, *ptch1*, and *Gli-1*; our qPCR data corroborated the cDNA data in showing that pretreatment Shh mRNA levels were significantly lower in pathCR cases compared to <pathCR cases (mean ±SD: 3.76±1.85 for pathCR vs 11.66±5.66 for <pathCR; p = 0.017; t-test). Expression of both *ptch* and *Gli-1* was up in non- responders, but not significantly (*ptch*, P=0.15; *Gli-1*, P=0.42). However, at the protein level, Gli-1 performs as well as Shh (see below). Gli-1 performed better than Shh in a new and larger sample size (see Table 2 below). Nuclear Gli-1 may be a better biomarker than Shh. These data suggest that upregulation of the Hh pathway is associated with chemoradiation resistant EACs and Gli-1 plays an important role.

Aberrant Hh Signaling is Associated with the Burden of Chemoradiation-Resistant EAC Cells. To validate our initial expression array findings and assess whether the pathway was activated (presence of nuclear Gli1) we determined the levels of Shh and Gli1 protein expression, using immunohistochemistry, in untreated cancer specimens obtained from 63 EAC patients treated with trimodality therapy. Shh expression was detected in all EACs, along with nuclear Gli1 in 59 (93.6%) cases. The labeling indices (LI) of Shh and Gli1 positive EAC cells varied [median LI (range): Shh = 0.20 (0.01-0.80); Gli1 = 0.45 (0.00-0.90)], however, within the same EAC, the levels of Shh and Gli1 co-expressions were significant (P<0.0001, r=0.485, Spearman test), thus confirming the functional activation of the pathway. Shh expression was usually clustered in distinct tumor patches surrounded by larger tumor fields expressing nuclear Gli1 staining. The presence of positive staining for cytoplasmic Shh and nuclear Gli1, indicating activation of the Hh pathway, was not statistically associated with age, cancer location, histopathologic grade, or clinical stage. Pretreatment expression levels of Shh and Gli1 were significantly lower in cancers sensitive to chemoradiation and achieving pathCR compared to chemoradiation (CTXRT)-resistant tumors (P<0.0001). Interestingly, the pretreatment labeling indices (LI) of both Shh and Gli1, proteins were significantly associated with the degree of residual cancer cells in the resected specimen.

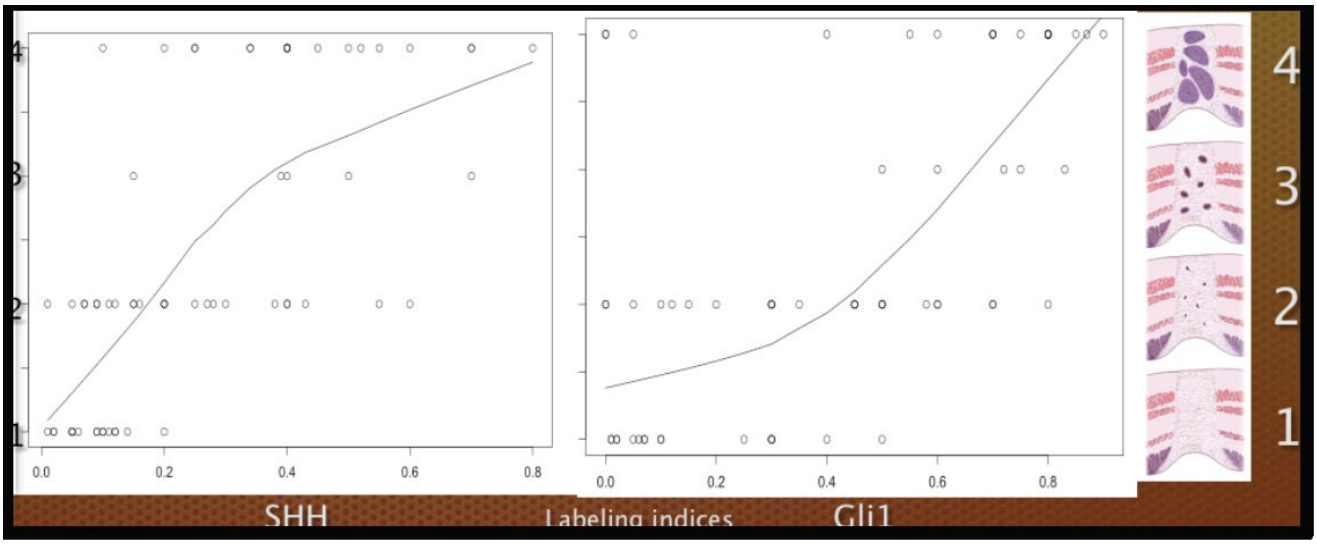


Figure **2**. LIs of Shh and Gli-1 correlate with the degree of resistance (right column shows resected specimens; 4 = highest resistance)

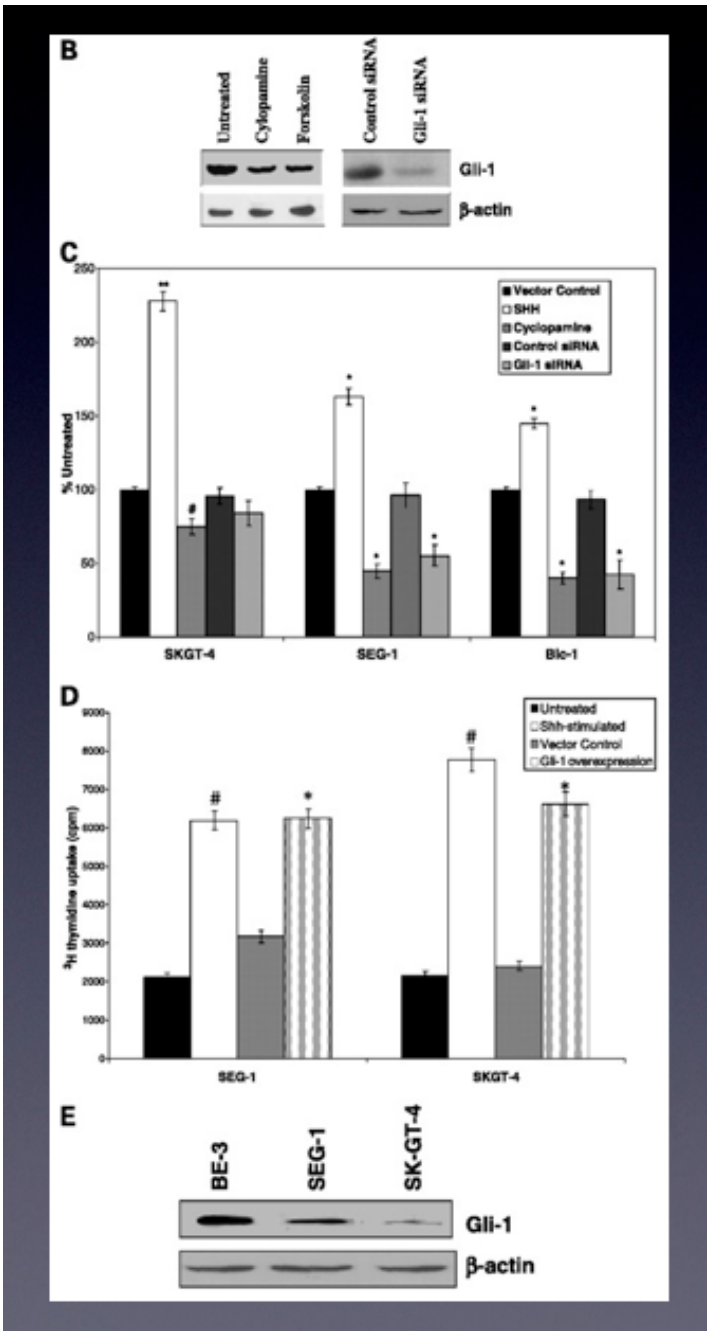
The post-treatment expression of Shh and Gli-1 (in 47 patients with <pathCR) was noted in 41 patients (87.2%) with resistant EACs.

Aberrant Hh is Associated with Poor Overall Survival (OS): In the univariate Cox proportional hazards model for OS, Shh and Gli-1 were significant prognosticators. However, pretreatment Shh was the strongest prognosticator with a relative risk of death increasing proportionally with higher Shh LIs (Hazard Ratio [HR]=14.6; 95% CI: 2.41–87.8; P=0.004), compared to pretreatment Gli-1 LIs (HR=3.93; 95% CI: 1.02–15.1; P=0.047). The Kaplan-Meier (KM) OS plots showed a difference between the two groups of patients using a cut-off value for Shh (P=0.01; log-rank test) or of Gli-1 (P=0.03). In a multivariate model, that included age, clinical stage, and tumor location, pretreatment Shh (cut-point=0.395) was the only independent prognosticator (HR=2.93; 95% CI: 1.19–7.24; P=0.02). However, our focus is on prediction of response (and not on OS). In addition, for prediction of response, Shh, Gli-1, or NF-kB individually has unsatisfactory sensitivity and specificity levels for clinical implementation. Thus we combined them to produce a signature (details below).

Hh Signaling Plays an Oncogenic Role in EAC *in vitro* and *in vivo*: Our reports[30] and by others[31] confirm that the majority of EAC cell lines and tumor tissues have high LIs for Shh/Gli-1. Hh signaling (constitutive/acquired) is important for growth/maintenance. Genetic knock down or inhibition by cyclosporine/GDC-0449 decreases proliferation, migration, invasion, and colony formation but increases apoptosis *in vitro* and decreases tumor growth *in vivo*.[30] An increase in proliferation (in SEG-1; previously considered EAC) treated with Shh and a decrease when treated with cyclopamine (Figure **3**) was observed.

Figure 3. Activation of Hh signaling induces proliferation and knock down Gli-1 decreases EAC

cell growth.



Reduced proliferation when treated with cyclopamine or Gli-1 siRNA compared to controls was noted. In contrast, increase in 3H-thymidine uptake in SEG-1 cells treated with Shh ($P<0.01$) or transiently overexpressing Gli-1 ($P<0.05$) compared to controls. Our recent data show that blocking Hh *in vivo* by GDC-0449 in a mouse xenograft reduced tumor growth (Figure **4**).

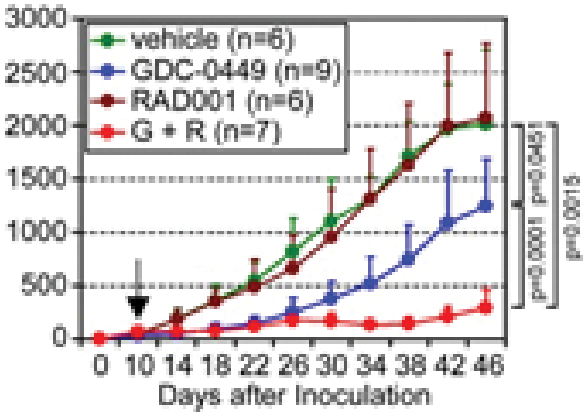


Figure 4. Inhibition of Hh by GDC-0449 decrease EAC tumor growth in vivo.

Hh Signaling Precedes Tumor Repopulation after Chemoradiation in Tumor Xenografts: We demonstrated that Shh and Gli-1 are among the "first responders" after chemoradiation injury and provide strong support for the clinical trial proposed.[32, 33]

Hh Mediated Chemoradiation Resistance and *in vivo* Inhibition to Overcome it: Our new data show that EAC cells with high Shh and nuclear Gli-1 are resistant to fluorouracil (FU) and docetaxel while cells with low Shh and Gli-1 are sensitive (Figure **5**). Inhibition of Hh by cyclopamine combined with docetaxel *in vivo* has dramatic results (Figure **6**). This supports the combination of Hh inhibition with standard chemoradiation approach in an enriched EAC patient population.

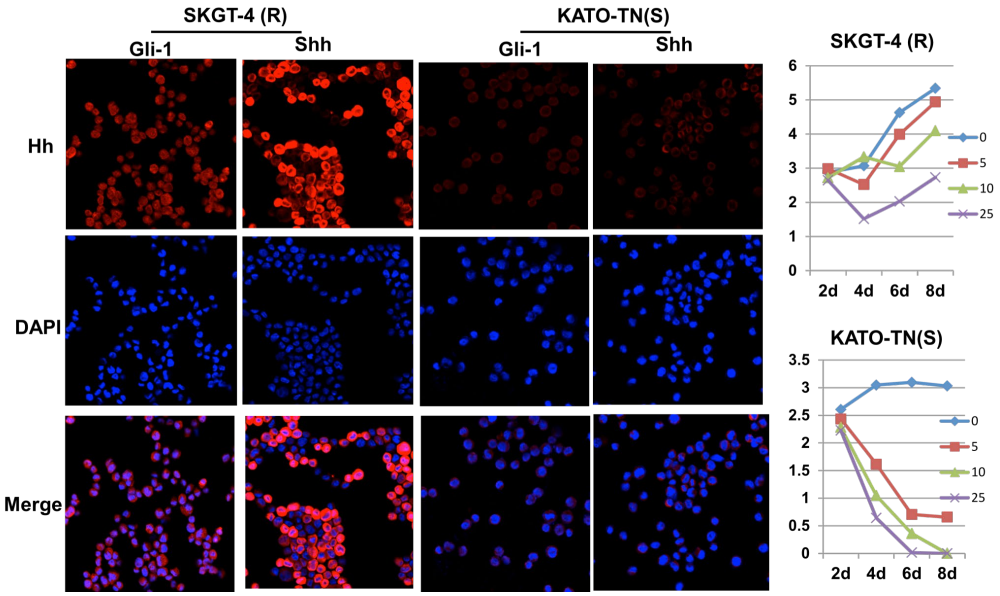


Figure 5. Hh signaling associated with chemotherapy resistance.

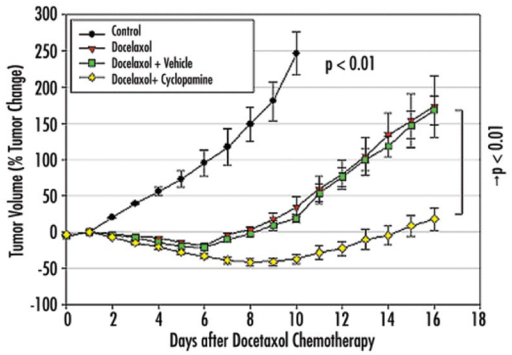


Figure 6. Hh inhibition by cyclopamine increased the effect of docetaxel on tumor growth delay (in vivo model).

Inhibition of Hh signaling (by drugs, siRNAs, or genetic knock downs) sensitized cells to radiation *in vitro*.[33] Knockdown of Gli-1 by siRNA greatly increased caspase 3 activity which increased further when radiation was added.[33]

Chemoradiation Resistance and Predictive Signature in EAC. The pathway analyses of cDNA array also indicated that NF-kB pathway was differentially upregulated in non-responders. Subsequent IHC studies on pre- and post chemoradiation cancer specimens confirmed NF-kB's role and its association with aggressive phenotype and resistance.[34, 35]

Combined Hh/NF-kB/Gli-1 Signature Predicts for pathCR after Chemoradiation: We analyzed NF-kB, Shh, and Gli-1 protein in untreated cancer of 60 EAC patients undergoing chemoradiation. No cut-points were used but only raw labeling indexes (LIs) of each of the 3 biomarkers for each patient was used to compute a single score that predicted response to therapy. A multiple logistic regression model was used to fit for the endpoint of pathCR with Gli-1, Shh, and NF-κB included as covariates. The goodness-of-fit was assessed by the method of Hosmer et al., where high p values indicate a well-calibrated model. The log-likelihood ratio test and Akaike information criteria was used for model selection. Based on the final fitted model, a linear Z score was computed for each patient. For example, if the final model contains all 3 biomarkers, the linear Z score for patient i can be

expressed as follows: $Z_i = \hat{\beta}_0 + \hat{\beta}x_{i1} + \hat{\beta}_2 x_{i2} + \hat{\beta}_3 x_{i3}$, where xi1, xi2 and xi3 are the expression level of Gli-1, Shh, and NF-κB for the patient i, respectively, and i=1, 2, ..., 60. Also, the predicted probability of having pathCR for each patient i was computed based on the above formula.

The functional relationship between the linear Z scores and the predicted probabilities of pathCR were plotted and the true pathCR patients were identified on this plot. The area under the receiver operating characteristic (ROC) curve, i.e., AUC, was used to assess the discriminating ability of the fitted model. Figure **7** shows the ROC curve for the discovery set. All pathCR patients had a similar score and only a few non-pathCR patients had a score approaching that for pathCR. This indicates that scoring model is highly accurate for pathCR prediction. The ROC curve for the fitted multiple logistic regression model, confirmed the high accuracy of the prediction, with corrected AUC values after cross-validation and bootstrapping were 0.943 and 0.940, respectively.

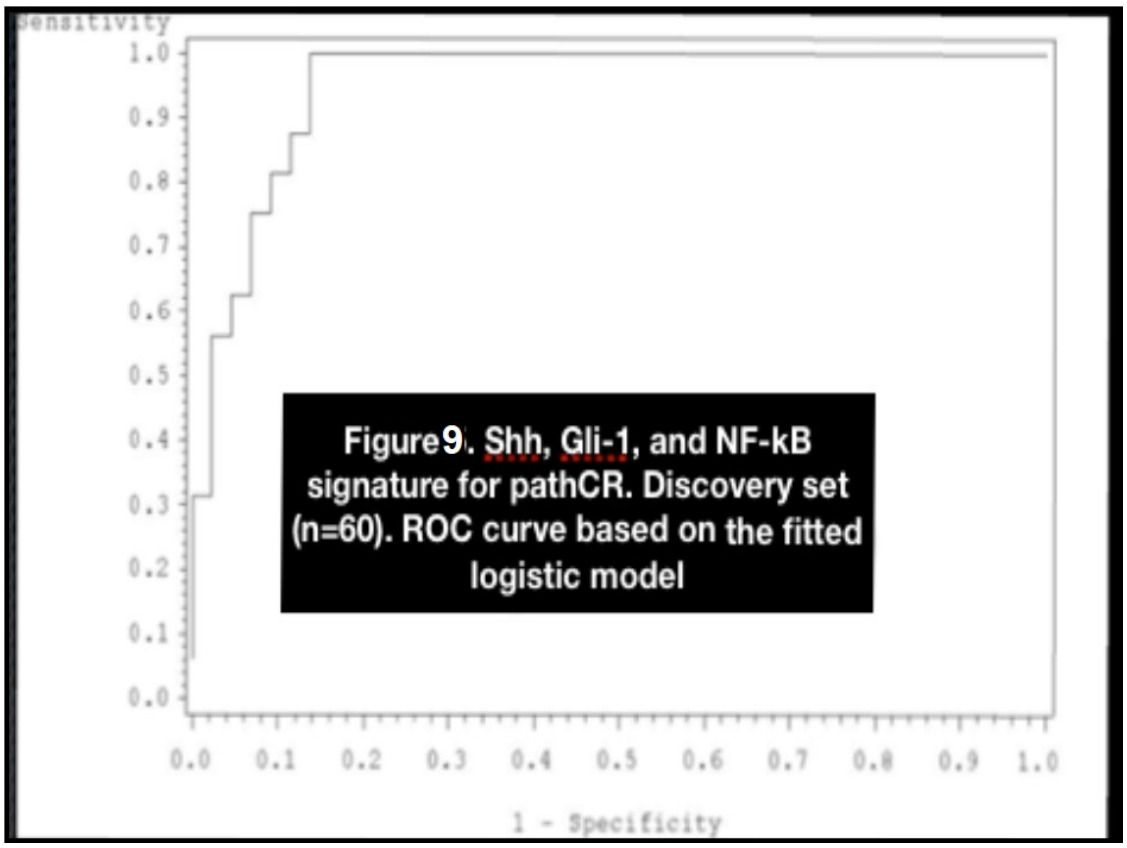


Figure 9. Shh, Gli-1, and NF-kB signature for pathCR. Discovery set (n=60). ROC curve based on the fitted logistic model

Table 1. Multivariate logistic regression model for pathCR (n=167) ALDH-1 can replace Shh

Model	Covariates	AUC for ROC curve	95% Confidence Interval
1	Shh, Gli-1, NFkB	0.97	0.95 – 0.99
2	Aldh-1, Gli-1, NFkB	0.97	0.95 – 0.99

Validation of the Signature: In a unique set of 167 patients who had chemoradiation followed by surgery, untreated cancer was stained for Shh, Gli-1, and NF-kB (and others). Table **1** shows the AUCs with CIs and Figure **8** shows the ROC curve. Table **2** shows Likelihood Estimates for all biomarkers studied. These data suggest that the signature has been retrospectively validated and is ready for prospective validation as proposed.

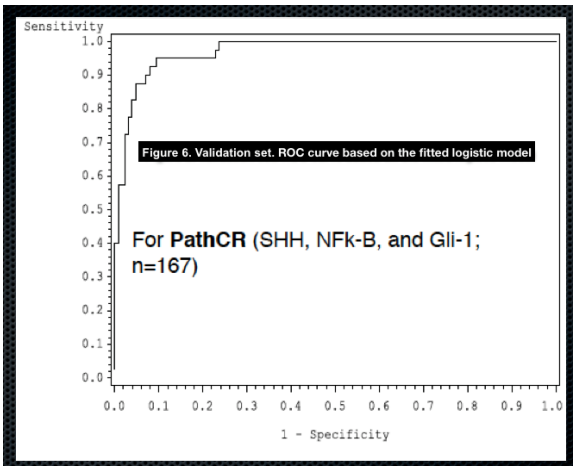


Figure **8**. Validation set. ROC curve

Table **2.** Analysis of Maximum Likelihood Estimates (pathCR; n=167)

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	P-value
Intercept	1	3.7340	1.0794	11.9659	0.0005
NF-kB	1	-23.0420	7.2880	9.9960	0.0016
Shh	1	-2.9563	3.5455	0.6953	0.4044
Gli-1	1	-12.8989	4.8566	7.0540	0.0079
ALDH1	1	-1.8985	3.0851	0.3787	0.5383
ABCB1	1	0.1640	1.5002	0.0119	0.9130
Survivin	1	0.9916	1.3300	0.5558	0.4559
BCRP	1	-1.8382	1.3346	1.8970	0.1684

Gli-1 Expression and Response to Chemoradiation (**new**): In 227 patients (we have clinical response data on all patients), the median Gli-1 LI was 0.25 (range, 0-0.95). In pathCR patients (n=56), the median was 0.05 (range, 0-0.5), and in <pathCR patients (n=171), the median was 0.35 (range, 0-0.95). These data suggest a trend that the higher the Gli-1 LI, the more resistant the cancer. We are choosing ≥5% LI for enrichment based on these numbers. There is no other trial or publication using Gli-1 for enrichment. For our trial, it is very likely that the median Gli-1 LI will be around 0.25. It is highly unlikely that most tumors will have Gli-1 LI of ≤10%.

2.2 Investigational Agent

2.2.1 TALADEGIB

The Hh signaling pathway is a crucial mediator of embryogenesis[37]. Signaling is initiated by the binding of the secreted morphogen, Hh, to its receptor, patched 1 (Ptch1). In the unbound state, Ptch1 inhibits SMO, a G-protein coupled phosphoprotein receptor, by preventing its localization to the cell surface; however, in the presence of the Hh ligand, the Hh-Ptch1 complex is internalized and the repression of Ptch1 on SMO is relieved. Surface localization of SMO is thought to initiate a signaling cascade, leading to the activation of the glioma-associated (Gli) family of zinc finger transcription factors, many of which are involved in proliferation, survival, and angiogenesis.

Aberrant activation of the Hh pathway in cancers is caused by mutations in the pathway or through Hh overexpression, termed either ligand-independent or ligand-dependent, respectively (for reviews, see [38, 39] Past studies have identified mutations in the Hh receptor components, Ptch1 or SMO in basal cell carcinoma (BCC) and medulloblastoma, resulting in constitutive pathway activation.[40, 41] Excessive or inappropriate expression of the Hh ligand has been found in a significant proportion of patients with sporadic cancers of the gastrointestinal tract, pancreas, lung and prostate, suggesting that disruption of Hh signal transduction could potentially be beneficial in a broad array of tumor types[42-45] Evidence suggests that antagonism of excessive Hh signaling may provide a route to unique mechanism-based anticancer therapies, blocking tumor growth and stimulating tumor regression without toxic effects on normal adjacent tissue.[45]

TALADEGIB is a small-molecule antagonist of the Hh signal pathway (Investigator's Brochure, 2013). Specifically, TALADEGIB binds to and inhibits SMO, blocking Hh signal transduction. *In vitro* and *in vivo* preclinical studies have demonstrated inhibition of Hh signaling following TALADEGIB administration. TALADEGIB has demonstrated efficacy against a variety of primary human tumor xenografts, including colorectal cancer (CRC) and pancreatic adenocarcinoma, and tumor cell-line xenograft models. Inhibition of Hh signaling in xenograft models has been correlated with a decrease in tumor growth.

Nonclinical Studies

Metabolism
Evaluation of Cytochrome P450 Enzyme Induction Potential in Rats and Dogs

The potential of TALADEGIB to induce total hepatic cytochrome P450 (CYP) was evaluated in male and female rats and dogs following oral drug administration daily for 28 days. TALADEGIB did not induce hepatic total CYP enzyme levels in rats or dogs.

In Vivo Metabolism

The metabolism of [14C]TALADEGIB was examined in male and female Sprague Dawley rats and male beagle dogs. In the plasma of rats and dogs, parent compound and active metabolite LSN2941091 (amide N-desmethyl, M75) were the major circulating components. Plasma half-lives in dogs were significantly longer (5.5- to 20-fold) for radioactivity than for either TALADEGIB or LSN2941091 following both oral and IV administration.

Excretion

In single-dose excretion studies with [14C]TALADEGIB in rats and dogs, the radioactivity was primarily eliminated in the feces (89% to 90% in male and female rats, and 90% in male dogs) following oral administration. In addition, studies of oral administration of TALADEGIB to bile duct-cannulated rats and IV administration of TALADEGIB to dogs demonstrated that biliary excretion plays a major role in the elimination of TALADEGIB.

Toxicity Summary

Long-term studies to evaluate the carcinogenic potential of TALADEGIB have not been conducted. TALADEGIB was negative in a bacterial mutation (Ames) assay. Animal studies to evaluate the impairment of fertility have not been conducted; however, TALADEGIB did cause testicular and epididymal injury, and uterine atrophy in repeat-dose studies in animals. These effects would be expected to impair fertility.

TALADEGIB treatment produced anticipated embryo-fetal lethality in female Sprague-Dawley rats when administered orally. Given the understanding of the pivotal role Hh signaling plays in early embryonic development, as well as published reports of malformations in offspring of pregnant animals exposed to Hh inhibitors during pregnancy, the potential for embryotoxicity due to TALADEGIB exposure in pregnant woman is of particular concern. Therefore, administration of TALADEGIB to patients poses a significant risk to a pregnant mother and unborn child.

Clinical Experience

As of 12 August 2013, a total of 66 patients in Studies HHBB and HHBE were treated with at least 1 dose of study drug. TALADEGIB has also been administered in a total of 36 healthy volunteers (Studies HHBG and HHBF). Study HHBD is being closed due to lack of accrual.

Table 3 lists the planned, ongoing, and completed studies at the time of this update.

Table 3. Planned, Ongoing, and Completed Studies for TALADEGIB

Study	Design	Population	Status

HHBB	Phase 1 FHD monotherapy Part A dose escalation QD 50-600 mg Part B dose escalation BID (optional) Part C dose confirmation all comers 400 mg Part D dose confirmation BCC patients only 400 mg	Parts A to C: advanced cancer Part D: locally advanced or metastatic BCC patients	Closed to enrollment Part A: MTD defined as 400 mg QD 84 patients: 50 mg: 3 pts 100 mg: 6 pts 200 mg: 3 pts 400 mg: 6 pts 600 mg: 7 pts Part B: not enrolled Part C: 400 mg: 19 pts Part D: 400 mg: 40 pts
HHBE	Phase 1b/2 TALADEGIB 100-400 mg and Carboplatin + Etoposide	Extensive stage SCLC patients	Part A ongoing 24 patients: 100 mg: 6 pts 200 mg: 6 pts 400 mg: 12 pts
HHBH	Phase 1 Study 100-400 mg	Japanese patients with advanced solid tumors	Ongoing 15 patients: 100 mg: 3 pts 200 mg: 3 pts 400 mg: 9 pts
HHBJ	Phase 1 Study	Japanese patients with SCLC	Planned
HHBG	SAD study Part A dose escalation 50-400 mg Part B formulation/food/PPI effect 100mg	Healthy subjects	Completed N=30
HHBF	^{14}C metabolism study 100 mg	Healthy subjects	Completed N=6

Abbreviations: BCC = basal cell carcinoma; BID = twice daily; FHD = first human dose; FPV = first patient visit; MTD = maximum tolerated dose; QD = once daily; PPI = proton pump inhibitor; pt = patient; SAD = single ascending dose; SCLC = small cell lung cancer.

Pharmacokinetics in Patients

Interim PK data are available for 56 patients across a dose range of 50 mg to 600 mg. Plasma concentration versus time profiles were analyzed using the noncompartmental method of analysis to determine the PK parameters for TALADEGIB and its equipotent metabolite LSN3185556.

After single- and multiple-dose administration, TALADEGIB levels reach Cmax after approximately 3 hours, with food appearing to have no impact on rate or extent of absorption. The mean value for the apparent clearance is 7.6 L/hr (99% coefficient of variation [CV]); indicating that TALADEGIB is slowly eliminated from the plasma. The mean value for the apparent volume of distribution is 215 L (65% CV). The mean TALADEGIB terminal half-life (t 1/2) across all doses is estimated to be around 23 hours (range, 5 to 80, 70% CV). The exposure ratio between metabolite and parent was calculated from the AUC 0-24hr and is, on average, approximately 2.7 (range, 0.8 to 5.4, 39% CV). From 50- to 100-mg doses, both C max and exposure don't seem to

increase based on the limited data available. However, in doses ranging from 100 to 600 mg, TALADEGIB and LSN3185556 seem to exhibit dose-proportionality increases in exposure (C max and AUC on Day 1 and Day 15). In vitro data suggest that both TALADEGIB and LSN3185556 are 100% metabolized by CYP3A4. Given this patient population, with its concomitant medications and varying stages of disease, this could explain at least some of the PK variability.

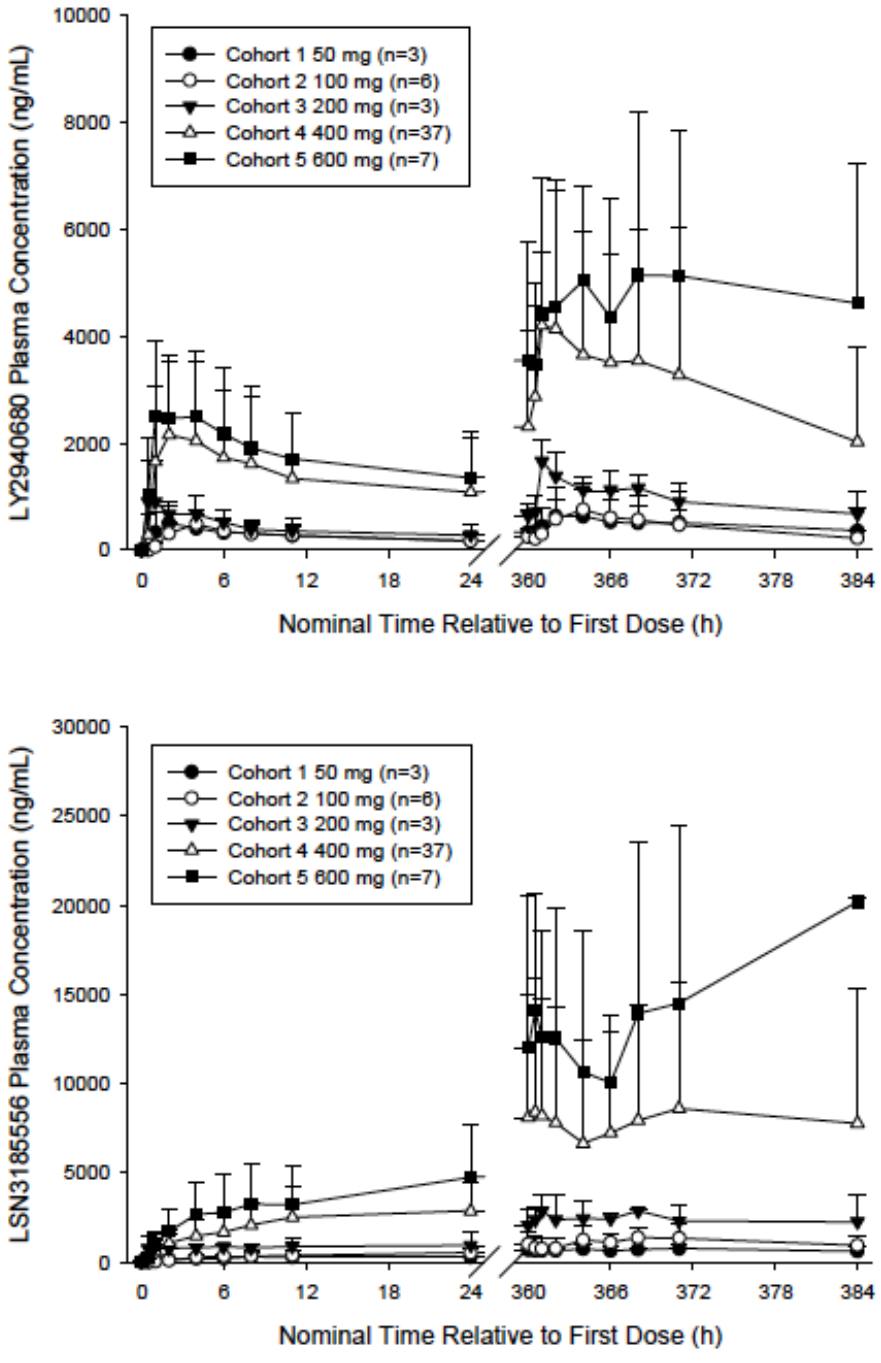


Figure 9 Arithmetic mean and SD plots per dose level for TALADEGIB (top) and LSN3185556 (bottom) following single- and multiple-dose administration of TALADEGIB from Study HHBB

(patient data).

Pharmacokinetics in Healthy Volunteers
In Study HHBF, 6 healthy subjects have been enrolled to study the disposition of radioactivity and TALADEGIB and its metabolite LSN3185556. The median time of maximum observed drug concentration (tmax) was observed at 1.5 hours postdose for TALADEGIB and 2.5 hours for metabolite LSN3185556. The mean t1/2 was 12.5 hours for TALADEGIB and 11.9 hours for metabolite LSN3185556. LSN3185556 was a major metabolite, with a ratio of metabolite to parent drug MR AUC0-72hr, MR AUC0-∞, and MR Cmax of 3.60, 3.68, and 1.49, respectively.

TALADEGIB represents less than 1% of the excreted dose, indicating extensive metabolism with metabolites excreted primarily in the feces with minor urinary excretion of metabolites. In Study HHBG part A, 12 healthy subjects have been enrolled to explore the safety and tolerability features of TALADEGIB administration following single doses (50 to 400 mg). The median tmax across all doses was estimated to be approximately 2 hours for TALADEGIB and 9 hours for LSN3185556. The mean t 1/2 across all doses was estimated to be approximately 16 hours for TALADEGIB and 17 hours for LSN3185556. These PK properties are similar to those described in patients from the ongoing Phase 1 Study HHBB.

In Study HHBG part B, 14 healthy subjects have been enrolled to receive a single 100-mg TALADEGIB dose. A minimal increase in both AUC and Cmax was seen following tablet administration compared to capsule following a 100-mg dose. This increase was approximately 9% to 10% for AUC and 29% to 33% for Cmax for both TALADEGIB and LSN3185556. In the fed state, a slightly lower Cmax was observed for both TALADEGIB and LSN3185556 (38% and 27%, respectively). There was no change in AUC for TALADEGIB and a 9% decrease in AUC for LSN3185556 in the fed state. With co-administration of a proton pump inhibitor (PPI), the AUC showed a slight decrease (3% and 11% for AUC of TALADEGIB and LSN3185556, respectively). There was a slight increase in the Cmax (8%) for parent and no change in the Cmax for metabolite following PPI administration. Overall, these results indicate that the tablet formulation of TALADEGIB should provide similar exposure to the capsule formulation, and that there are negligible food and gastric pH effects on the PK parameters following single 100-mg doses of this formulation. Therefore, no restrictions in food nor elevated gastric pH from a PPI are needed for TALADEGIB administration.

Pharmacodynamics (PD) in Patients
Despite variability in the PK results, the PD data demonstrate at least 50% inhibition of Gli1 in skin, which was observed in the majority of patients in Study HHBB. This is what was targeted from the preclinical studies, where efficacy has been associated with survival and a 50% PD response in skin for at least 4 hours

Clinical Metabolism
Results from a human radiolabeled metabolism study (Study HHBF) indicate that TALADEGIB is extensively metabolized. In plasma from 0-72 hours, the mean exposure to TALADEGIB was 11% of the total plasma radioactivity. The major plasma metabolite is the free base of the active metabolite LSN2941091 (amide N-desmethyl, M75 or LSN3185556). This is the same metabolite that was found in plasma from both rats and dogs.

The mean total recovery of radioactivity in excreta was 97.3%, with 82.7% of the dose recovered in feces and 14.6% recovered in urine. The major excretory metabolite is M43, an amide N-demethylation plus piperidine di-oxidation of parent. Metabolite M43 represented a combined total of approximately 40% of the radioactive dose, primarily excreted in feces. Parent TALADEGIB represented less than 1% of the dose in the excreta, indicating extensive metabolism. The major biotransformation pathways observed in humans included amide N-demethylation and piperidine ring oxidation.

Safety and Efficacy
Safety
Clinical Pharmacology Studies
There have been 2 studies of TALADEGIB in healthy subjects (Studies HHBG and HHBF).

Study I4J-MC-HHBG
Study HHBG was a single ascending dose and relative bioavailability study of TALADEGIB in healthy subjects. During Part A, 16 (2 male, 14 female) healthy subjects received TALADEGIB in a dose escalation manner (50, 100, 200, and 400 mg). During Part B, 14 female healthy subjects received 100 mg of TALADEGIB on 4 separate occasions. The incidence of treatment emergent adverse events (TEAEs) was comparable across the different treatments, and all TEAEs reported during the study were mild in severity. The most frequently reported TEAEs considered to be related to study drug were headache and nausea. Headache was reported by 11 of the 30 subjects (37%) following dosing with TALADEGIB and by 2 of the 9 subjects (22%) following dosing with placebo. Nausea was reported by 5 of the 30 subjects (17%) following dosing with TALADEGIB and by 1 of the 9 subjects (11%) following dosing with placebo. All other TEAEs were reported by no more than 3 subjects (≤10%). There were no clinically significant changes in the clinical laboratory results, vital signs, or 12-lead ECGs during the study.

Study I4J-MC-HHBF
Study HHBF was a disposition study of [14C]-TALADEGIB following oral administration in healthy subjects. Six female subjects received a single 100-mg oral dose of TALADEGIB containing approximately 100 mCi of [14C]-TALADEGIB. There were 5 TEAEs reported, all of which were mild in severity. The most frequently reported TEAEs considered to be related to study drug were headache and diarrhea. There were no clinically significant changes in the clinical laboratory results, vital signs, or ECGs during the study.

Safety Data across Clinical Studies in Cancer Patients

Study I4J-MC-HHBB
The 58 patients exposed to TALADEGIB in the Phase 1 Study HHBB have received doses ranging from 50 mg to 600 mg daily. Data included are from electronic case report forms (eCRFs) from these patients through 12 August 2013, as well as any reported SAEs considered related to TALADEGIB administration as of 12 August 2013. In these patients, hyponatremia (n=1) was the only Grade 4 TEAE considered possibly related to TALADEGIB by the investigators; this event was also reported as serious. In addition, Grade 3 nausea/vomiting (n=1) and Grade 3 syncope (n=1) were reported as drug-related serious adverse events (SAEs) by the investigators. Other

Grade 3 TEAEs occurring at least once and considered possibly related to TALADEGIB by the investigators were fatigue (n=3); nausea, dysgeusia, vomiting, asthenia, and muscle spasms (n=2 each); and maculopapular skin rash, diarrhea, myalgia, and decreased body weight (n=1 each). The most frequently reported TEAEs considered possibly related to TALADEGIB were fatigue (41.4%), dysgeusia (41.4%), nausea (39.7%), vomiting (31.0%), and decreased appetite (including 1 report of early satiety) (31.0%). In addition, treatment-emergent muscle spasms (including 1 report of tetany) considered possibly related to TALADEGIB occurred in 27.8% of treated patients. Alopecia (20.7%), rash (including rash maculopapular, dermatitis, and urticaria) (17.2%), diarrhea (15.5%), asthenia (13.8%), myalgia (13.8%), and decreased body weight (10.3%) were also TEAEs considered possibly related to TALADEGIB at a frequency of ≥10%. Of the 58 patients treated, 51 (87.9%) patients experienced at least 1 treatment-emergent event considered possibly related to TALADEGIB by the investigators (all grades). The MTD in Study HHBB was defined as 400 mg QD in Part A (dose escalation) of Study HHBB; enrollment is ongoing in Part D (BCC-specific MTD expansion) of this study. The MTD of 400 mg QD was determined based on the observation of DLTs at the 600-mg QD dose level in 2 individual patients. The first DLT, observed at the 600-mg QD dose level, was Grade 3 maculopapular rash. The second DLT, also observed at the 600-mg QD dose level, was determined by the investigator based on numerous Grade 2 toxicities occurring within the same patient during Cycle 1 (confusion, nausea, and dehydration). Of note, another DLT was reported during the dose escalation phase (Part A) of Study HHBB: hyponatremia (initially Grade 3, but worsened to Grade 4) at the 100-mg QD dose level.

Study I4J-MC-HHBE

Study HHBE is a dose-escalation study of TALADEGIB in combination with the standard of care chemotherapy doublet of carboplatin and etoposide in patients with extensive stage small cell lung cancer. Patients have received doses ranging from 100 to 200 mg daily. Data included are from the eCRFs from 8 patients through 12 August 2013, as well as any reported SAEs considered related to TALADEGIB administration as of 12 August 2013. Compared to the TALADEGIB monotherapy Study HHBB, Study HHBE showed a relative increase in adverse events due to hemotoxicity. Besides Grade 3 and 4 neutropenia possibly related to TALADEGIB (n=1 each), there was one serious report of Grade 5 enterocolitis (with febrile neutropenia) considered possibly related to TALADEGIB. This event was also considered a DLT for Cohort 1 (100 mg QD TALADEGIB in combination with carboplatin and etoposide). Given the known hemotoxicity profiles of carboplatin and etoposide, the additional role of TALADEGIB in these adverse events is unclear at this stage of development. Other drug-related TEAEs reported in Study HHBE were generally consistent with the monotherapy data reported from the 58 patients treated in Study HHBB.

Table 4. Adverse Drug Reactions Occurring in ≥10% of Patients in Study HHBB (N=58)

Event	All Grades (%)	Grade ≥3
Dysgeusia	46.2	2.2
Fatigue	44.1	4.3
Nausea	43.0	2.2
Muscle spasms (including 1 report of tetany)	37.6	5.4
Decreased appetite (including 3 reports of early satiety)	33.3	0.0
Alopecia	32.3	0.0
Vomiting	30.1	2.2
Decreased Weight	25.8	1.1
Myalgia	18.3	2.2
Diarrhea	18.3	2.2
Rash (including rash maculopapular, dermatitis acneiform, and urticarial)	16.1	1.1
Asthenia	10.8	2.2
Constipation	11.8	0.0

Patients will be only counted once in each preferred term but may be counted in more than one preferred term.

Sources:

Home/lillyce/ prd / ly2940680 /integrations / programs_stat / tfl_output / hhbb_smdrad12.rtf; Home / lillyce / prd / ly2940680 /integrations / programs_stat / tfl_output / hhbb_lspaed1.rtf

Supplier: Ignyta Inc. (Ignyta)

How supplied: The drug product is supplied for clinical trial use as 100-mg tablets.

Administration: Oral

Storage and Stability: The drug substance is stable when stored at room temperature. .The drug product should be stored according to instructions on the label.

2.3 Other Agents

Carboplatin

Carboplatin is a second-generation platinum compound with a broad spectrum of antineoplastic properties. Carboplatin contains a platinum atom complexed with two ammonia groups and a cyclobutane-dicarboxyl residue. This agent is activated intracellularly to form reactive platinum complexes that bind to nucleophilic groups such as GC-rich sites in DNA, thereby inducing intrastrand and interstrand DNA cross-links, as well as DNA-protein cross-links. These carboplatin-induced DNA and protein effects result in apoptosis and cell growth inhibition. This agent possesses tumoricidal activity similar to that of its parent compound, cisplatin, but is more stable and less toxic.

Supplier: Carboplatin will be obtained from a commercial source. Please refer to the current FDA-approved package insert provided with the drug for complete instructions for preparation, handling and storage of the drug and information about possible side effects.

Solution preparation: Preparation is per the package insert.

Administration: Intravenous

Storage and Stability: Store the unopened vials at 25°C (77°F); excursions permitted from 15°-30°C (59°-86°F). Protect unopened vials from light. Solutions for infusion should be discarded 8 hours after preparation. Unopened vials of carboplatin are stable for the life indicated on the package when stored at 25°C (77°F); excursions permitted from 15°-30°C (59°-86°F). Protect from light. When prepared as directed, carboplatin solutions are stable for 8 hours at room temperature (25°C). Since no antibacterial preservative is contained in the formulation, it is recommended that carboplatin solutions be discarded 8 hours after dilution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Paclitaxel (Taxol®)

Paclitaxel (Taxol®) is an antineoplastic agent belonging to the taxoid family. Paclitaxel Injection Concentrate is a sterile, pyrogen-free, non-aqueous, clear yellow to brownish-yellow viscous solution and is available in single-dose vials containing 20 mg (0.5 mL) or 80 mg (2 mL) paclitaxel (anhydrous), with an accompanying sterile, non-pyrogenic, diluent (13% ethanol in Water for Injection) vial. Each mL contains 40 mg paclitaxel (anhydrous) and 1040 mg polysorbate 80.

Premedications are required and include: dexamethasone 10 mg IV, hydrocortisone 100 mg IV, and diphenhydramine hydrochloride 25 mg IV.

Supplier: Paclitaxel will be obtained from a commercial source. Please refer to the current FDA-approved package insert provided with the drug for complete instructions for preparation, handling and storage of the drug and information about possible side effects.

Solution preparation: Preparation is per the Paclitaxel package insert.

Administration: Paclitaxel is administered through an intravenous infusion over 1 hour.

Storage and Stability: Store the vials in original cartons between 20°–25° C (68°–77° F). Retain in the original package to protect from light. Unopened vials of TAXOL (paclitaxel) Injection are stable until the date indicated on the package when stored between 20°–25° C (68°–77° F), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Upon refrigeration, components in the TAXOL vial may precipitate, but will redissolve upon reaching room temperature with little or no agitation. There is no impact on product quality under these circumstances. If the solution remains cloudy or if an insoluble precipitate is noted, the vial should be discarded. Solutions for infusion prepared as recommended are stable at ambient temperature (approximately 25° C) and lighting conditions for up to 27 hours.

2.4 Rationale

Rationale for the Study: Localized EAC is a virulent cancer. Most patients are treated with chemoradiation followed by surgery. Most patients' tumors are chemoradiation resistant. Patients whose tumors are chemoradiation sensitive tend to have a longer survival than those with tumors that are chemoradiation resistant. Therefore, overcoming chemoradiation resistance is of considerable importance. Varying degrees of Hh signaling are observed in most EAC. Hh signaling is also correlated with resistance to therapy in EAC patients and in preclinical studies in EAC.[32, 36] Therefore, treatment with TALADEGIB, a Hh signaling inhibitor, would be of considerable interest as it may partially or completely overcome chemoradiation resistance in some patients.

Rationale for Choosing This Combination: Chemoradiation therapy has been shown to be superior to radiation alone in patients with carcinoma of the esophagus.[46] Therefore, chemoradiation is preferred over radiation alone. However, there is no single combination of cytotoxic drug that has shown to be superior.[16, 47-49] We have previously reported on the schedule of taxane and fluoropyrimidines used concurrently with radiation therapy. [13, 50-52] In this study, we are using paclitaxel as our preferred taxane. This combination of weekly paclitaxel and weekly carboplatin with radiation has become the current standard approach for this group of patients with the publication of the CROSS trial. [53] The proposed radiation dose of 50.4 Gy in 28 fractions is the national standard.

2.5 Correlative Studies Background

Hedgehog (Hh) Signaling: Hh signaling is critical for growth and differentiation during embryonic development.[54] Secreted Hh molecules (Sonic [Shh], Desert [Dhh], and Indian [Ihh]) bind and inhibit the cell surface receptor Patched (Ptch). This inhibition relieves the Ptch-mediated suppression of the transmembrane protein Smoothened (Smo), leading to intracellular events that cause activation (i.e. proteolytic processing) and nuclear translocation of the Gli family of transcription factors (Gli-1, -2 and -3).[55] The three Gli proteins vary considerably. While both Gli-2 and -3 have transcriptional activation and repression properties,[56] Gli-1 is a strong regulator of Hh targets and is itself a transcriptional target of the mammalian Hh pathway.[57] Transcriptional targets of Gli-1 include genes implicated in cell cycle control, cell adhesion, signal transduction, vascularization, apoptosis, stem cell maintenance and Ptch itself.[58-60]

The Hh pathway is involved in the maintenance of the normal stem cells and production of the progeny that differentiate into specialized cell lineages.[59, 60] The different Hh ligand proteins, Shh, Ihh, and Dhh, appear to control independent stem cell pools. In physiologic conditions, the

Hh pathway is silent in the quiescent stem cells to become activated by other signaling, notably the Wnt/b-catenin axis[61-63] during the regeneration processes. Once activated, the Hh pathway is responsible for sustained gradient-dependent proliferation and progeny differentiation.

Alterations of the Hh Pathway in Cancer: The initial observation of Gli-1 involvement in glioma development[64] suggested the importance of Hh in tumorigenesis. Later, a definite link between the Hh pathway and cancer was established by the identification of heterozygote germline mutations affecting PTCH and abnormal activation of the Hh pathway in basal cell carcinoma, rhabdomyosarcoma, and neural tumors.[65] Several *in vitro* and *in vivo* studies suggest constitutive, ligand-dependent, activation of Hh signaling in cancers of the GI tract.[42, 43, 66] Most GI cancers lack mutations in the Hh pathway genes, suggesting pathway activation distinct from other cancers.

Studies suggest that Hh signaling promotes cellular proliferation by opposing signals to physiologic growth arrest. Most of the Hh pathway effects are transcriptionally mediated by the Gli family through the upregulation of cell cycle regulatory targets (i.e. N-Myc, D- and E-type cyclins, or phosphatase CDC25B) and anti-apoptotic proteins (i.e. Bcl2).[67] Additionally, other transcriptional targets of Hh include growth factors and pro-angiogenic factors, such as VEGF, EGF, and PDGF.[68] Thus, the deregulation of Hh pathway in cancer cells can contribute to cancer maintenance and progression by enhancing cell cycle alterations and production of cancer growth factors**.**

Relevance of autocrine versus paracrine Hh signaling in cancer: Several studies have concluded that human cancer cell lines constitutively express high levels of Hh ligands and Smo leading to proliferation in response to autocrine ligand-mediated receptor activation.[42, 69, 70] Recent data strongly suggest that autocrine pathway activation is also important for the maintenance of cancer stem cells.[71, 72] However, paracrine signaling between tissue epithelial cells and associated stroma is critically important during normal development,[73] and the notion that human cell lines depend on autocrine signaling for proliferation has been challenged.[74] Yauch et al. directly investigated the relative importance of autocrine versus paracrine signaling in determining tumor responsiveness to their specific Hh pathway antagonist *in vitro* and *in vivo*.[75] They concluded that the effects of chemical pathway antagonists on proliferation observed in the previous studies[42] were probably due to off-target effects of the drugs and that the responses of xenografts tracked more closely with disruption of tumor-stromal paracrine Hh signaling.[75]

It seems likely to us that both conclusions are correct and that the importance of autocrine versus paracrine signaling is highly tumor context-dependent. The Yauch et al.'s[75] data do not rule out the possibility that autocrine Hh activation is generally required for the maintenance of cancer stem cells (which typically represent a small percentage of cells) and could be upregulated by inflammation and/or stress. Our data in preclinical models and patient tumors demonstrate that Hh pathway components are strongly induced by exposure to chemoradiation, raising the possibility that conventional therapies will stimulate autocrine and/or paracrine pathway activation in EAC.

3. PATIENT SELECTION

Except as indicated below, the inclusion and exclusion criteria will be the same for Phase IB and Phase II parts of this trial.

3.1 Inclusion Criteria

3.1.1 Histologically or cytologically confirmed adenocarcinoma of the esophagus or

gastroesophageal junction (EAC).

3.1.2 Male or female patients whose age is ≥18 years (Because nonclinical studies suggest that TALADEGIB may adversely affect the development and maintenance of teeth, bones, and growth plates in a pediatric population, children are excluded from this study. In addition, EAC is extremely rare in children).

3.1.3 Localized EAC and its baseline clinical stage determined as: T2-T3N0 or T1-3N+. Imaging studies suspicious for metastases must be followed with a negative biopsy before a patient can enter the study.

3.1.4 Patients with malignant celiac nodes are eligible if the primary lesion is in the mid-thoracic or distal thoracic esophagus or it is involving the gastroesophageal junction.

3.1.5 Tumor must have labeling index of ≥5% of the nuclear Gli-1 (integral biomarker) performed in the MD Anderson Cancer Center CLIA laboratory for patient to be eligible in this trial (if enough archival tissue is not available to determine labeling index, patient must agree to a biopsy to determine eligibility for the study).

3.1.6 Tumor may not extend >4 cm below the gastroesophageal junction.

3.1.7 ECOG performance status 0 or 1.

3.1.8 All patients must be willing to provide research tumor tissue for biomarker studies at baseline, from archival tumor tissue or through endoscopy if sufficient archival tumor tissue is not available. All patients must also allow biomarker studies on the tissue obtained through surgery to remove the primary cancer.

3.1.9 Phase II only**:** Patients volunteering for the Phase II part of the protocol must be willing to undergo a research endoscopy for tissue collection on day 8 (+/- 2 days) from the beginning of therapy.

3.1.10: Absolute neutrophil count ≥ 1500/mm^3; Platelets ≥100,000/mm^3; Hemoglobin ≥8 g/dL; Serum creatinine ≤ 2 x Upper Limit of Normal (ULN); ALT and AST ≤2.5 x ULN; Serum bilirubin ≤1.5 x ULN.

3.1.11 Patient must be able to comprehend the approved consent document and have the willingness to sign it. The patient prior to enrollment and the administration of any protocol-specific therapy must sign the consent document.

3.1.12 Willingness and ability to comply with study procedures and follow-up examinations.

3.1.13 Must be considered medically fit for operation as determined by multidisciplinary evaluation.

3.1.14 Effects of TALADEGIB on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because Hh signal pathway inhibitors as well as other

therapeutic agents used in this trial are known to be teratogenic, males and females with reproductive potential must agree to use 2 forms of medically approved contraceptive precautions (see Appendix B) and for at least 6 months following the last dose of biochemoradiation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

Women of childbearing potential are defined as follows:

- Having regular menstrual cycles
- Has amenorrhea, irregular menstrual cycles or using a contraceptive method that precludes withdrawal bleeding.
- Have had a tubal ligation.

Women are considered not to be of childbearing potential for the following reasons:

- Had hysterectomy and/or bilateral oophorectomy.
- Post-menopausal defined by amenorrhea for at least 1 year in a woman >45 years old.

3.1.15 Females with childbearing potential must have a negative serum pregnancy test within 14 days prior to treatment start.

3.2 Exclusion Criteria

3.2.1 Baseline clinical stage of T1N0 or inoperable T4 (unequivocal organ involvement) are to be excluded.

3.2.2 Unequivocal metastatic tumor at baseline.

3.2.3 Tracheo-esophageal (TE) fistula or direct invasion into the tracheo-bronchial mucosa. A bronchoscopy (biopsy and cytology should be performed) is required to exclude TE fistula or tracheo-bronchial involvement in patients with a tumor located at <26 cm from the incisors.

3.2.4 Cervical esophageal cancer will not be entered in this study.

3.2.5 Any prior chemotherapy, surgery, or radiotherapy for EAC.

3.2.6 Prior mediastinal irradiation (for any reason).

3.2.7 Clinically significant ulcerative colitis, inflammatory bowel disease, or partial or complete small bowel obstruction are to be excluded.

3.2.8 Malabsorption syndrome or other condition that would interfere with intestinal absorption are excluded.

3.2.9 Pregnant or nursing females are to be excluded. Pregnant women are excluded from this study because TALADEGIB is a Hh pathway-inhibiting agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with TALADEGIB, breastfeeding should be discontinued if the mother is treated with TALADEGIB. These potential risks may also apply to other agents used in this study.

3.2.10 Presence of other significant cancer(s) or history of other significant cancer(s) within the last 3 years (patients who have been cancer-free for 3 years, or have a history of completely resected non-melanoma skin cancer or successfully treated in situ carcinoma of the cervix are eligible).

3.2.11 Known active viral or other chronic types hepatitides (Hepatitis B, C) or cirrhosis.

3.2.12 Uncontrolled concurrent illness including, but not limited to: serious uncontrolled infection, symptomatic congestive heart failure (CHF), unstable angina pectoris, cardiac arrhythmia that interfere with blood pressure, uncontrolled diabetes, or psychiatric illness/social situations that would limit compliance with the study requirements.

3.2.13 Patients with uncontrolled hypocalcemia, hypomagnesemia, hyponatremia or hypokalemia defined as less than the lower limit of normal for the institution, despite adequate electrolyte supplementation.

3.2.14 Patients who are receiving concurrent non-protocol anti-cancer therapy (chemotherapy, radiation therapy, surgery, immunotherapy, hormonal therapy, targeted therapy, biologic therapy, or tumor embolization) are to be excluded.

3.2.15 Patients may not be receiving any other investigational agents.

3.2.16 Patients with known hypersensitivity to taxanes or platinums are to be excluded.

3.2.17 Patients taking medications with narrow therapeutic indices that are metabolized by cytochrome P450 (CYP450), including warfarin sodium (Coumadin®) are ineligible. Patients on strong CYP3A inhibitors will also be excluded.

3.2.18 Known HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with TALADEGIB. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.2.19 Any other conditions or circumstances that would, in the opinion of the Investigator, make the patient unsuitable for participation in the study.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Patients can begin study therapy only after the pretreatment evaluation is completed and eligibility criteria are met. Patients are registered prior to any protocol therapy through the Clinical Oncology Research (CORe) system developed and maintained by the Office of Protocol Research at MD Anderson Cancer Center.

Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. All patients will need intravenous (IV) access established prior to the beginning of therapy.

Reported adverse events and potential risks for TALADEGIB and weekly paclitaxel, weekly carboplatin, and radiation therapy are described in Section 7. Appropriate dose modifications for TALADEGIB and weekly paclitaxel, weekly carboplatin, radiation therapy are described in Section 6.

5.2 Phase I B

The purpose of phase IB study is to establish safety of TALADEGIB with standard chemoradiation. The starting dose is based on summarized data (and data in communication with Ignyta). This starting dose has resulted in objective responses in patients with locally advanced and metastatic BCC and has resulted in inhibition of HH signaling as measured by Gli-1 inhibition. We anticipate entering 6 patients to be recruited to complete the study as follows:

Table 5A

Phase 1B REGIMEN DESCRIPTION					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
TALADEGIB	No premedication. Can take with or without food.	400 mg (fixed dose)	PO in A.M.	Daily for 38 days, starting on first chemoradiation day	NA
Paclitaxel*	Decadron up to 10 mg i.v., hydrocortisone 100 mg i.v., diphehydramine hydrochloride 25 mg i.v.	50 mg/m^2/week IV	IV 3 hr	First radiation day of each week x 5 doses (usually on Mondays)	
Carboplatin	None	AUC 2mg/ml/min	IV 2hr	First radiation day of each week x 5 doses (usually on Mondays)	
Radiation	None	1.8 Gy	External Beam	On 28 consecutive weekdays	

* Paclitaxel to be given before Carboplatin.

In the event of unexpected toxicities (i.e. More than 1 DLT in the first 6 patients) in the first 6 patients, we will study additional 6 patients but TALADEGIB 200 mg will be administered every day.

5.3 Definition of Dose-Limiting Toxicity

TOXICITY	DLT CRITERIA
Cardiac disorders	Cardiac toxicity CTCAE Grade ≥ 3 or cardiac event that is symptomatic or requires medical intervention
	Congestive heart failure Grade ≥ 2
Vascular disorders/ Hypertension	Persistent hypertension CTCAE Grade ≥ 3 requiring more than one drug or more intensive therapy than previously
Skin and subcutaneous tissue disorders: Rash and/or photosensitivity[a]	Rash or photosensitivity CTCAE Grade 3 for > 7 consecutive days despite skin toxicity treatment
	Rash or photosensitivity CTCAE Grade 4
Metabolism and nutrition disorders:	Hyperglycemia Grade 2 (FPG 200 - 249 mg/dL; 11.2 - 13.8 mmol/L) (confirmed with a repeat FPG within 24 hrs) that does not resolve to Grade 0 (< 140 mg/dL; < 7.8 mmol/L) within 14 consecutive days (after

TOXICITY	DLT CRITERIA
Hyperglycemia[b]	initiation of oral anti-diabetic treatment)
. . .	Hyperglycemia Grade 3 (FPG 250 – 399 mg/dL; 13.9 - 22.2 mmol/L) (confirmed with a repeat FPG within 24 hrs) for > 7 consecutive days despite oral anti-diabetic treatment
	Hyperglycemia Grade 4 (FPG ≥ 400 mg/dL; ≥ 22.3 mmol/L)
	Hyperglycemia leading to diabetic keto-acidosis, hospitalization for IV. insulin infusion, or non-ketotic coma
GI disorders[a]	Diarrhea CTCAE Grade ≥ 3 for ≥ 48 hrs, despite the use of anti-diarrhea therapy
.	Nausea/vomiting CTCAE Grade ≥ 3 for ≥ 48 hrs, despite the use of anti- emetic therapy
	Pancreatitis CTCAE Grade ≥ 3
Investigations[c]	Blood total bilirubin CTCAE Grade 2 for > 7 consecutive days
	Blood total bilirubin CTCAE Grade ≥ 3
	Serum Creatine Kinase (CK) > 3
	AST or ALT CTCAE Grade 3 in conjunction with blood bilirubin[d] CTCAE Grade ≥ 2 of any duration
	AST or ALT CTCAE Grade 3 for > 7 consecutive days
	AST or ALT CTCAE Grade 4
	Serum alkaline phosphatase CTCAE Grade 4
	Serum lipase and/or serum amylase (asymptomatic) CTCAE Grade 3 > 7 consecutive days
	Serum lipase and/or serum amylase (asymptomatic) CTCAE Grade 4
	Serum creatinine CTCAE Grade ≥ 3
	Hypomagnesaemia CTCAE Grade 3 for > 3 consecutive days and not correctable with supplements, or symptomatic
	Hypomagnesaemia CTCAE Grade 4
	Hypokalemia CTCAE Grade 3 for > 3 consecutive days and not correctable with supplements, or symptomatic
	Hypokalemia CTCAE Grade 4
	Platelet count CTCAE Grade 3 for > 7 consecutive days and/or with signs of Bleeding
	Platelet count CTCAE Grade 4
	ANC CTCAE Grade 3 for > 7 consecutive days
	ANC CTCAE Grade 4
	Febrile neutropenia CTCAE Grade ≥ 3

TOXICITY	DLT CRITERIA
Other hematologic & non-hematologic toxicities	Any other CTCAE Grade ≥ 3 toxicity except: Lymphocyte count decreased (lymphopenia) CTCAE Grade ≥ 3 unless clinically significant Grade 3 Fatigue, Dehydration, Hiccups, Dysgeusia (as these are cumulative toxicities seen commonly with chemoradiation, and will only be considered DLTs if they do not resolve with appropriate supportive care)

[a] Patients will not initially receive prophylactic treatment for skin toxicity or nausea/vomiting. However, prophylactic treatment may be initiated in all patients at the dose level where these toxicities have been observed and in all further patients if at least 1 patient has experienced skin toxicity or nausea/vomiting CTCAE Grade ≥3 or if at least 2 patients experienced skin toxicity or nausea/vomiting CTCAE Grade ≥ 2. However anti-emetics may be applied for treatment if the patient has experienced nausea/vomiting CTCAE Grade ≥ 1, at the discretion of the physician.

[b] Not according to CTCAE v4.0. Of note: Hyperglycemia occurring during corticosteroids administration will be only considered DLT if not resolved within 2 days after the end of corticosteroid treatment.

[c] For any hepatic toxicity CTCAE Grade 4, or CTCAE Grade 3 that does not resolve within 7 days to CTCAE Grade ≥ 1 (or CTCAE Grade ≥ 2 if liver infiltration with tumor present), an abdominal CT scan should be performed to assess if it is related to disease progression.

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.4 Phase II

Following completion of the Phase IB part of the study, the phase II trial will be initiated.

The phase II trial will be performed in two sequential steps: Step 1- single agent TALADEGIB for 7 days followed by TALADEGIB plus chemoradiation (in 27 patients) and Step 2- TALADEGIB plus chemoradiation (in 27 patients). The patients will not be randomized but rather entered sequentially in to step 1 until full, followed by enrollment of 27 patients in to step 2.

Table 5B

Phase II REGIMEN DESCRIPTION					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
TALADEGIB	No premedication. Can take with or without food.	As determined in Phase Ib mg (fixed dose)	PO in A.M.	**FOR STEP 1 ONLY. TALADEGIB given alone orally for 7 days prior to start of bio-chemo radiation as described below.**	NA
TALADEGIB	No premedication. Can take with or without food.	As determined in Phase Ib mg (fixed dose)	PO in A.M.	Step 1 and Step 2: Daily for 38 days, starting on first chemoradiation day	NA
Paclitaxel*	Decadron up to 10 mg i.v., hydrocortisone 100 mg i.v., diphehydramine hydrochloride 25 mg i.v.	50 mg/m^2/week IV	IV 3 hr	First radiation day of each week x 5 doses (usually on Mondays)	
Carboplatin	None	AUC 2mg/ml/min	IV 2hr	First radiation day of each week x 5 doses (usually on Mondays)	
Radiation	None	1.8 Gy	External Beam	On 28 consecutive weekdays	

* Paclitaxel to be given before Carboplatin.

The purpose of the first step is to assess pharmacodynamic changes after TALADEGIB alone but also after TALADEGIB plus chemoradiation. We do believe the first step will not result in a different pathCR rate; therefore, all patients will be assessed similarly for pathCR (including those treated at the MTD in phase IB cohort).

In the Phase II part of the trial, patients will undergo a research upper endoscopy on day 8 (+/- 2 days) from the start of treatment (either starting with TALADEGIB alone or biochemoradiation) to obtain pictures of the tumor for comparison with the baseline and to obtain tumor tissue for biomarker assessment. The research endoscopy will be performed only if deemed safe by the attending endoscopist. Standard procedures will be followed.

Treatment with TALADEGIB alone followed by biochemoradiation: Patients in Step 1 will receive TALADEGIB 400 mg PO in A.M. daily for 7 days and then patient will start biochemoradiation therapy as described in Table 5B.

Treatment with biochemoradiation: Patients in Step 2 will proceed with biochemoradiation therapy as described in section Table 5B.

5.5 Treatment Information

5.5.1 TALADEGIB

TALADEGIB is an oral drug. Patients should take TALADEGIB in the morning at approximately the same time every day, with or without food. If a patient misses a dose, he or she should be instructed not to take or make up that dose and to resume dosing with the next scheduled dose. Missed doses should not be made up. Patients will be instructed to bring all unused tablets and their medication diary to each study visit for assessment of compliance.

- Patients will be instructed not to share their supply of TALADEGIB.

5.5.2 Other Agents

Please see section 2.3

5.5.3 **Radiation therapy**

Definition of Target Volumes

GTV Gross tumor volume is all known gross disease as demonstrated on the planning CT, and modified as deemed necessary based on PET and other clinical studies.

iGTV GTV plus margin for tumor motion. A maximum intensity projection (MIP) of the 4D CT image data set will be reconstructed. MIP images will display the maximum extent of tumor motion during respiration. The delineated MIP-GTV will be compared with the actual motion of the GTV on each of the 10 phases and modified, if necessary, to encompass the extent of motion of the GTV (hence iGTV). The iGTV will be modified as deemed necessary based on PET and other clinical studies.

CTV Clinical target volume is the subclinical involvement around the GTV. The CTV is the GTV plus a 30-mm margin superiorly and inferiorly. CTV for lateral margin will be individually drawn by the radiation oncologist, usually 8 mm laterally for microextensions of the tumor.

ITV Internal target volume is the envelope of the CTV during the time of irradiation, thus accounting for intrafractional motion.

PTV Planning target volume is ITV plus a margin to ensure that the prescribed dose is actually delivered to the ITV. This margin accounts for variations in treatment delivery, including variations in setup between treatments. The ITV is expanded by 5 mm to generate the PTV in accordance with our current clinical practice. The PTV is relevant to photon planning.

Radiation will be either photon or proton.

Radiation Doses

The total radiation dose for the tumor target will be 50.4 Gy at 1.8 Gy per fraction and a total of 28 fractions. Radiation will be once a day, 5 fractions per week.

100% of the ITV will be covered by the prescribed dose. Greater than or equal to 95% of the prescribed dose to the ITV will be acceptable if 100% cannot be reached.

95% of PTV should receive prescribed dose.

Tolerance Limits for Critical Structures

Normal lung (right lung + left lung - GTV): V10 ≤ 40%; and mean lung dose (MLD) ≤ 20Gy.

Brachial Plexus: Minimum dose to 2% highest dose volume ≤ 60 GY. Doses ≤ 66 Gy will be considered as minor deviation.

Spinal cord: Maximum dose to 2% highest dose volume ≤ 50 Gy.

Heart: 1/3 volume must be ≤ 60 Gy, 2/3 < 45 Gy and whole volume ≤ 30 GY.

Liver: 1/3 volume must be ≤ 35 Gy and the whole liver must be ≤ 25 Gy.

Kidney: 1/3 of one kidney should receive < 17 Gy. One kidney should be kept out of the radiation beams if possible.

Simulation, Immobilization, Image-Guided Radiation

Simulation: All simulations will be done on CT scanners capable of acquiring 4D CT image data sets.

For proton planning, only CTs, which have been calibrated for protons, should be used.

Each patient will be positioned in an immobilization device in the treatment position on a flat table.

The imaging session will consist of acquisition of a free-breathing treatment planning CT image data set and a 4D CT image data set.

The pretreatment diagnostic PET/CT should be performed together with 4-D CT simulation if at all possible.

Localization image studies with the patient in the treatment position will be obtained.

Treatment Planning

Critical structures

The normal tissues including the right lung, the left lung, the esophagus, the heart, liver, kidneys, brachial plexus, and the spinal cord need to be contoured in their entirety.

The lungs are expected to be the primary dose-limiting structure. Every effort should be made to keep the total lung dose (defined as the volume of both lungs minus the GTV in 50% phase of 4-D CT) to a minimum.

The tolerance doses to various organs shown in Table 6 and 7 below are to be used as guidelines. Physicians/dosimetrists should make every effort not to exceed these tolerance levels. (All tolerance doses are assumed to be delivered at 2 Gy/fx.)

Table 6

Incidence of radiation pneumonitis *(%)*					
Quartile	GTV *(cc)*	Mean Dose *(Gy)*	% of Ipsilateral Lung Receiving >20 Gy	% of Total Lung Receiving >20 Gy	V_{eff}
>Grade 2					
1st	32	20	7	8	0
2nd	12	21	10	23	23
3rd	27	25	38	29	25
4th	27	29	42	33	45
>Grade 3					
1st	11	10	7	8	0
2nd	6	11	0	0	5
3rd	3	8	21	19	14
4th	20	24	25	27	26

Table 7

Radiation Tolerance Dose			
Organ	Volume	TD 5/5	End Point
Spinal Cord	5 cm	50 Gy	Myelitis
	10 cm	50 Gy	Myelitis
	20cm	47 Gy	Myelitis
Heart	1/3	50 Gy	Clinical Pericarditis
	2/3	45 Gy	Clinical Pericarditis
	3/3	40 Gy	Clinical Pericarditis
Liver	½	35 Gy	Clinical Hepatitis
	2/2	30 Gy	Clinical Hepatitis
Kidney	1/3	17 Gy	Clinical nephritis

Therapy Interruptions

If interruption of therapy (up to three weeks) becomes necessary, radiation therapy should be completed to the prescribed doses. Total number of fractions and elapsed days should be carefully reported. If an interruption of more than two weeks is necessary, resumption of treatment is at the discretion of the radiation oncology chairs. The patient will be considered a major deviation, but follow-up will be continued.

If the patient develops > grade 3 RT-related toxicity, radiation therapy and biochemotherapy should be withheld. Treatment can resume once grade 3 RT-related toxicity is no longer present. If a patient develops grade 3 esophagitis in the last week of treatment (i.e. with 5 or fewer radiation treatments remaining), radiation therapy (but not chemotherapy) may continue at the discretion of the treating physician.

Criteria for Toxicity

Acute and late toxicity related to radiation therapy include fatigue, myelosuppression, skin erythema, subcutaneous fibrosis, esophagitis, carditis, myelitis, acute radiation pneumonitis and late pulmonary fibrosis, and esophageal stricture.

Acute toxicity monitoring: Acute (<90 days from RT start) side effects of radiation therapy will be documented using the revised NCI Common Toxicity Criteria, Version 4.0, which can be downloaded from the CTEP home page (http://ctep.info.nih.gov).

Late toxicity monitoring: Late (>90 days since RT start or persisting beyond 90 days) post-treatment complications will be evaluated and graded according to the RTOG Late Effects Radiation Morbidity Criteria (RTOG.org).

5.5.4 Surgery

All patients will be allowed to recover from biochemoradiotherapy for 5-6 weeks and then undergo a complete preoperative evaluation. If there is no evidence of metastatic cancer, and patient is still medically fit for surgery, then an operation will be attempted.

Details of Surgery

After adequate preoperative evaluation, patients that are judged to be physiologically capable of undergoing resection are viewed as having a resectable lesion, and are without evidence of metastatic disease (M1b) will undergo surgery. Patients will understand the risks of surgical resection. A feeding jejunostomy will be put in place for postoperative nutritional support. A diagnostic laparoscopy with peritoneal washings examined for cytology may be appropriate for patients with locally advanced gastroesophageal junction tumors prior to embarking on open esophagectomy.

Lymphadenectomy

Patients will undergo esophagectomy with removal of local-regional lymph nodes. It is recommended that resections of distal esophageal or gastroesophageal junction tumors include lymph nodes at levels 7-20 according to the AJCC lymph node station chart if deemed appropriate under clinical circumstances. Level 10 would be omitted unless clinically indicated.

Patients with mid to upper thoracic esophageal lesions should also undergo paratracheal lymph node sampling as deemed appropriate based on the level of the lesion and surrounding regional lymph nodes. Sampling of level 1, supraclavicular lymph nodes is recommended when clinically indicated.

Reconstruction

Reconstruction will be completed in the chest or neck using stomach, small bowel, or colon as a conduit to re-establish gastrointestinal continuity.

Approach to the resection may be via transthoracic, transhiatal, three-field, or minimally invasive technique. The recommendations for surgical technique will depend upon the location of the lesion and surgeon/patient preference.

Description of Surgical Techniques and Risks
Transthoracic

Patients who undergo transthoracic esophagectomy will have incisions in the abdomen and chest. Recommended lymphadenectomy and feeding jejunostomy will be performed during the abdominal portion of the procedure. Thoracotomy will be performed for mobilization of the esophagus and intrathoracic lymph node sampling. A proximal and distal resection margin of

≥4cm is preferable, and pleural/pericardial resection may be required to obtain negative radial margins. Gastrointestinal continuity will be re-established in the chest with the chosen conduit.

Transhiatal

Patients will undergo esophagectomy similar to the technique described above though lacking a thoracic incision. The esophagus and intrathoracic lymph nodes are removed through incisions in the abdomen and neck. Reconstruction will be performed in the neck via the cervical incision.

Three-field Approach

A more extensive procedure that is recommended when an extensive lymph node dissection is desired or when greater exposure is required to complete the resection of the primary tumor with adequate proximal margins. The reconstruction is performed in the neck. Incisions are performed in the neck, chest and abdomen.

Minimally Invasive Esophagectomy

Surgeons may recommend a minimally invasive approach to esophageal resection. This involves laparoscopy and possible thoracoscopy to achieve removal of the esophagus and reconstruction. Additionally, an incision in the left neck may be made to complete the pull-up procedure.

Internally the operation is equivalent to the standard, open procedure, a lymph node dissection is recommended and the stomach is used to reconstruct gastrointestinal continuity. The risks of the procedure are similar to a standard esophagectomy with the additional risk or understanding that a minimally invasive procedure may have to be converted to an open technique in certain circumstances. Typically there is also an increase in operative time.

Risks
Risks of the procedure include but are not limited to: bleeding, internal or external wound healing complications (i.e. infection, hernia), pneumonia, cardiac arrhythmia, swallowing dysfunction, aspiration, hoarseness (temporary or permanent), gastrointestinal dysfunction (dumping, nausea, regurgitation, dysmotility, stricture, anorexia, weight loss, abdominal pain, reflux), anastomotic leak, graft loss, need for re-operation (acute or remedial), pain, and sepsis.

The operation carries approximately a 4% risk of mortality within the operative and immediate (30 days) post-operative period.

5.6 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of TALADEGIB with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. In addition, patients and their caregivers should be provided the patient information sheet (see Appendix C) describing potential interactions of TALADEGIB with other drugs and other remedies and medications.

5.6.1
TALADEGIB

- TALADEGIB and its major metabolite, LSN3185556 have both been evaluated for inhibition of CYP enzymes. The table below shows the results of the in vitro interaction studies. The CYP3A enzyme is predicted to be the enzyme most sensitive to inhibition by both compounds, where inhibition constant (Ki) values were 15 and 11 μM for TALADEGIB and LSN3185556, respectively.
- A substrate depletion approach with recombinant human CYPs predicted that CYP3A4 was the primary enzyme responsible for hepatic CYP-mediated clearance of both TALADEGIB and LSN3185556. Thus strong CYP3A4 inhibitors should be excluded when co-administering TALADEGIB.

Inhibition of the Catalytic Activities of CYP Enzymes by TALADEGIB and LSN3185556 in Human Liver Microsomes

Isoform	**TALADEGIB K_i or IC_{50} (μM)**	**LSN3185556 K_i or IC_{50} (μM)**
CYP3A (midazolam)	15	11
CYP2D6	99	53
CYP2C19	18	31
CYP2C9	22	14
CYP2C8	29	11
CYP2B6	17	41
CYP1A2	(>200)	(>200)

Abbreviations: CYP = cytochrome P450; IC_{50} = half-maximal inhibitory concentration; K_i = inhibition binding constant.

Use of medications or food that may interfere with the metabolism of TALADEGIB is prohibited, including ketoconazole and fresh-squeezed grapefruit juice.

5.7 Duration of Therapy

The duration of biochemoradiation therapy for the Phase IB part will be 5.5 weeks (chemotherapy for 25 days, TALADEGIB for 38 days, and radiation for 28 days in 28 fractions). The duration of therapy in the Phase II Step 1 will be 45 days (TALADEGIB for 45 days, radiation for 28 days, and chemotherapy for 25 days). In Phase II Step 2 it will be 38 days (chemotherapy for 25 days, TALADEGIB for 38 days, and radiation for 28 days in 28 fractions).
Therapy may be discontinued under the following circumstances:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse events(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.8 Duration of Follow Up

For the purpose of assessing toxicity in phase IB, all patients will be seen in the clinic weekly, continuing for two weeks after the completion of biochemoradiation. Phase II patients will also be monitored closely in the first two weeks after the completion of biochemoradiation but will not be necessarily seen in the clinic. Patients will be telephone-interviewed weekly and seen if necessary. The information acquired during the interview will be documented in writing.

Upon completion of the above evaluations, patients will be followed for 6 months after the 30 day safety evaluation or until death, whichever occurs first. Patients removed from the study for adverse events will be followed until resolution or stabilization of the adverse event.

5.9 Criteria for Removal from Treatment

Patients will be removed from treatment when any of the criteria listed in Section 5.7 and the reason(s) will be documented in patients' medical record.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose modification during biochemoradiation:

TALADEGIB: We do not anticipate any additional side effects in patients receiving the recommended 400 mg/day dose. However, if a DLT TALADEGIB-related toxicity during biochemoradiation is observed, then TALADEGIB will be administered every day at half the starting dose (200mg).

Carboplatin and Paclitaxel: Potential toxicities during chemoradiation include nausea, loss of appetite, vomiting, malaise, mucositis, hand-foot syndrome, dehydration, fatigue, and rarely myelosuppression and mild neuropathy. The following criteria will be used for modification of dose of carboplatin and paclitaxel.

Table 8

Reaction	Management of reactions
Renal:	
Creatinine ≤ 1.5 × upper limit of normal at day of treatment	Continue treatment
Creatinine > 1.5 × upper limit of normal	Establish intravenous infusion the evening preceding treatment at a rate to correct any volume deficits and produce a urine flow ≥ ml/h. Repeat serum creatinine value in the morning:
	≤ 1.5 × upper limit of normal → proceed treatment
	> 1.5 × upper limit of normal → stop chemotherapy, can re-start as clinically indicated
Gastrointestinal:	
Mucositis with oral ulcers or protracted vomiting despite antiemetic premedication	Delay chemotherapy one week

Neurologic:	
CTC grade ≤ 2	Continue therapy
CTC grade > 2	Stop chemotherapy, can re-start when resolved to 1 or less as clinically indicated
Cardiac:	
Asymptomatic bradycardia	Cardiology Consult: Continue therapy under continuous cardiac monitoring by cardiologist
First degree AV block	Cardiology Consult: Continue therapy under continuous cardiac monitoring by cardiologist
Symptomatic arrhythmia or AV block (except 1st degree) or other heart blocks	Stop chemotherapy, manage arrhythmia according to standard practice; patient goes off protocol

*Does not apply to alopecia, or grade 3 nausea and vomiting (if not adequately treated).

Patients requiring pre-emptive outpatient hydration will not be subjected to automatic dose modification.

Table 9

Granulocyte Nadir		Platelet Nadir	Dose Modification
>1,000	And	>75,000	No Change
≥500 but <1000	And/Or	≥50,000 but <75,000	No Change
<500	And/Or	<50,000	Hold weekly doses until the granulocyte count is >1,000 and platelet count is >100,000 then resume
Infection or bleeding directly related to myelosuppression			Hold weekly doses until the granulocyte count is >1,000 and platelet count is >100,000 and the infection has resolved then resume

Hypersensitivity reactions to Carboplatin

Carboplatin, as is the case with all platins, is associated with a low incidence of hypersensitivity reactions, usually after multiple doses. This can be manifest as bronchospasm, hypotension, and even hemolytic anemia.
. Cases of hypotension have been reported in spite of the use of glucocorticoids and antihistamine prophylaxis. The following treatment recommendations should be followed: Following a Grade 1 or 2 acute hypersensitivity reaction that is assessed as related to Carboplatin administration, the following premedication should be administered prior to each subsequent dose of Carboplatin (patients who have grade 3 or 4 acute hypersensitivity reactions should discontinue Carboplatin therapy): Dexamethasone 20 mg PO or IV, 12 and 6 hours prior to the dose; Dexamethasone 20 mg PO or IV, as well as diphenhydramine 50 mg IV, and one of the following: cimetidine 300 mg IV, ranitidine 50 mg IV, or famotidine 20 mg IV 30-60 minutes prior to Carboplatin administration.

Radiotherapy: See section 5.5.3

7. SAFETY MONITORING AND REPORTING

Protocol specific data and adverse events will be documented in the medical record and entered into the MD Anderson PDMS/CORe system. PDMS/CORe will be used as the electronic case report form (CRF) for this protocol.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

7.1 Adverse Events

Definitions and reporting

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the CRF Medical History. Adverse event monitoring should be continued for at least 30 days following the last dose of biochemoradiation. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs, symptoms or require therapy and are recorded as clinically significant by the physician.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity Grade (CTCAE Grade 1-4)
2. Its duration (Start and end dates or if continuing at the Safety Follow-up Visit)
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with Sequelae, fatal, unknown)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in Section 7.2

All adverse events should be treated appropriately. Such treatment may include changes in study treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other

medically required intervention. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the study treatment can be found in the protocol and TALADEGIB Investigators' Brochure. This information should be included in the patient informed consent and should be discussed with the patient during the study as needed.

7.2 Serious Adverse Events

Definition of Serious Adverse Event (SAE)

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

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- The MD Anderson electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

- Serious adverse events will be captured from the time of the patient signs consent until 30 days after the last dose of biochemoradiotherapy. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

- Events not considered to be serious are hospitalizations for the:

    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
    - Treatment which was elective or pre-planned, for existing condition that did not worsen.

Reporting to FDA

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

- It is the responsibility of the PI and the research team to ensure that serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

7.3 Communications between Investigator and Ignyta

Reporting responsibility: The principal investigator has the obligation to report all serious adverse events to Ignyta.

The investigator will submit a copy of the MD Anderson SAE form to Ignyta at the same time the form is submitted to the MD Anderson IND office. Follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation.

Pregnancies: Any pregnancy that occurs during study participation and for up to 6 months from the patients last dose must be reported. To ensure patient safety each pregnancy must be reported

to Ignyta within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and newborn complications.

In addition, each pregnancy occurring for a female partner of a male study participant must be reported while the patient is on study treatment and for up to 6 months after the patient's last dose. Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

7.4 Adverse Event List(s) for TALADEGIB

Table 10 Adverse Drug Reactions Occurring in ≥10% of Patients in Study HHBB (N=58)

Event	All Grades (%)	Grade ≥3
Dysgeusia	46.2	2.2
Fatigue	44.1	4.3
Nausea	43.0	2.2
Muscle spasms (including 1 report of tetany)	37.6	5.4
Decreased appetite (including 3 reports of early satiety)	33.3	0.0
Alopecia	32.3	0.0
Vomiting	30.1	2.2
Decreased Weight	25.8	1.1
Myalgia	18.3	2.2
Diarrhea	18.3	2.2
Rash (including rash maculopapular, dermatitis acneiform, and urticarial)	16.1	1.1
Asthenia	10.8	2.2
Constipation	11.8	0.0

Patients will be only counted once in each preferred term but may be counted in more than one preferred term.

Sources: Home / lillyce / prd / ly2940680 /integrations / programs_stat / tfl_output / hhbb_smdrad12.rtf; Home / lillyce / prd / ly2940680 /integrations / programs_stat / tfl_output / hhbb_lspaed1.rtf

Table 10 Adverse Drug Reactions Occurring in ≥10% of Patients in Study HHBB (N=58)

	All Grades (%)	Grade 3 and Above (%)
Fatigue	41.4	5.2
Dysgeusia	41.4	3.4
Nausea	39.7	3.4
Vomiting	31.0	3.4
Decreased appetite (including 1 report of early satiety)	31.0	0.0
Muscle spasms (including 1 report of tetany)	27.8	3.4
Alopecia	20.7	0.0
Diarrhea	15.5	1.7
Asthenia	13.8	3.4
Myalgia	13.8	1.7
Decreased weight	10.3	1.7
Rash (including rash maculopapular, dermatitis, and urticarial)	17.2	1.7

Sources:
home/lillyce/prd/ly2940680/integrations/araug2013/programs_stat/tfl_output/smdrad12.rtf;
home/lillyce/prd/ly2940680/integrations/araug2013/programs_stat/tfl_output / hhbb_lspaed1.rtf.

7.5 Adverse Event List(s) for paclitaxel and carboplatin

Please refer to the package inserts for the comprehensive list of adverse events. The following are commonly listed adverse events:

Table 11

Organ system	Adverse Event
Dermatology/Skin	XRT Dermatitis (chemoradiation-induced) Rash
Gastrointestinal	Dehydration Anorexia Esophagitis Diarrhea Constipation Nausea Vomiting Dyspepsia Mucositis/Stomatitis Dysphasia Weight loss
Pain	Odynophagia Abdominal Pain Substernal chest pain
Constitutional	Fatigue Insomnia Fever
Pulmonary	Dyspnea Pneumonitis/pulmonary infiltrates Hiccups
Blood/Bone Marrow	Anemia Leukopenia Granulocytopenia Thrombocytopenia
Infection	Febrile Neutropenia Infection Infection with normal neutrophil count
Metabolic	Hypokalemia Hypomagnesemia

7.6 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.
    - N/A

Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting. However, they still must be reported through the routine reporting mechanism:

Table 12

CTCAE Category	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
	Fatigue	3 or4	Yes/No	Chemoradiation	This is a cumulative toxicity seen in some patients in the last 10 days of therapy and it fully reversible.
	Dehydration	3 or 4	No	Chemoradiation	Oral intake decreases in patients in the last 2 weeks of chemoradiation. Outpatient hydration is often needed
	Hiccups	3 or 4	No	Chemoradiation	In less than 5% of patients, intractable hiccups occur as a result of cumulative effects of chemoradiation.

7.7 Data Confidentiality Plan

The Principal Investigator (PI) and study team will guard against any loss of confidentiality. Information about study participants will be kept confidential and managed under applicable laws, regulations and institutional requirements. The PI and all members of the research team have received training about maintaining confidentiality and safeguarding data. Study data will be stored on MD Anderson systems. These systems are secured by password protection, provide audit trails, and are backed up daily. Access to the systems are controlled by a sequence of individually assigned user identification codes and passwords, made available only to authorized personnel.

Study summary results will be provided to the National Cancer Institute in yearly reports. Study summary results will be provided to Ignytain quarterly reports and a final report upon study completion. No protected health information (PHI) will be included in these reports.

Only the principal investigator, study team, and other authorized entities identified in the IRB approved Consent/Authorization form will have access to view study PHI at MD Anderson.

Serious Adverse Event and pregnancy reports will be sent to Ignyta. These reports will use participants' protocol identification numbers, not names.

8. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

8.1 Biomarker Studies

Biomarker studies will be conducted in both phase Ib and phase II studies with the objectives to (1) confirm that TALADEGIB "hits" its target and produces the desired biological effects (apoptosis and/or growth inhibition) in tumors and (2) determine whether baseline autocrine and/or paracrine Hh pathway activation predicts clinical response to TALADEGIB. We will assess downregulation of Gli-1 and other ligand-dependent Gli-1 targets in post-treatment tissues, using the information obtained in Aims 1 and 2 to identify these markers. We will also use immunofluorescent TUNEL staining and laser scanning cytometry to compare pre- and post-treatment apoptosis levels as a pharmacodynamic measure of biological response using methods we have published previously.[76]

Expression studies: (1) We will conduct a pre- and post-treatment read-out of the pathway in EAC biopsies by determining the expression of Shh, Ih, Gli1 and Gli2, Patch 2 and sFRP1 mRNA using real-time qPCR. Endpoints: the primary end-points are to correlate the modulation of Hh signaling with therapy (single agent TALADEGIB and in combinations with CTXRT) and with clinical response. Secondary endpoint includes correlations with clinico-pathological variables.

(2) We will use gene expression data to refine and expand the pathway-associated markers listed in item #1 above and attempt to predict sensitivity or/and resistance to therapy in pre-treatment EAC biopsies using real-time qPCR.

(3) We will examine the levels and spatial localization of Shh, Ihh and Gli-1 using quantitative IHC in pre- and post-treatment cancer biopsies. Endpoints: to examine the spatial localization of these proteins, i.e. cancer cells vs. stromal component and to correlate the results to the Hh read-out mRNA expression profile by real-time qPCR.

(4) We will use fluorescent TUNEL and laser scanning cytometry (LSC) to measure levels of apoptosis in pre- and post-treatment biopsies as a measure of biological response. Biological response will be correlated with measures of Hh pathway inhibition and clinical response.

Mutation studies: Several *in vitro* and *in vivo* studies suggest a constitutive, ligand-dependent, activation of Hh signaling in cancers of the digestive tract without mutations in the pathway genes. To date, there are no systematic studies of the Hh genetic landscape is EAC. Thus, it cannot be excluded that ligand or Gli overexpressions, found in our own preliminary data, may be associated with genetic defects such as an increase copy numbers of their respective genes. This is a plausible hypothesis to be investigated in view of the reported gene amplification frequency at 7q36 and 12q13.2 chromosomal regions that are the locations for Shh and Gli-1 genes, respectively. Therefore, this is a unique opportunity to characterize the mutational and expression status of Hh pathway and correlate it to TALADEGIB clinical activity. We will assess: (1) Shh, Ihh, and Gli1 gene copy numbers using real-time qPCR; (2) PTCH1 loss of heterozygosity by multiplex capillary electrophoresis; (3) PTCH1, SMO and SUFU mutational analysis by gene sequencing in cancer specimens demonstrating Hh activation in the expression studies. *Assessment of proteins expression* will be performed using IHC techniques as previously described by our group.[21, 32, 34-36, 77-81] or others.

In consenting patients, residual tissue will be stored for potential future use after obtaining IRB approval.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy.

Table 13

Clinical Assessments	**Within 14 days of Registration**	**Within 30 days of Registration**	**Day 1**	**Day 8**	**Every 2 weeks during therapy**	**5-6 Weeks after completion of Bio-chemoradiation[µ]**
Informed Consent	X					
Gli-1 Testing		X				
Demographic Information	X					
Medical and Surgical History	X				X	X
Recording of Concurrent Illnesses	X					X
Disease History/ TNM Staging*	X					X
Height (screening only), Weight, and Vital Signs	X				X	X
BSA	X				X	X
ECOG Performance Status	X					X
Electrocardiogram (12-lead)		X				
EGD, EUS, biopsies, and photographs		X				X
Review inclusion/ exclusion criteria	X					
Physical Exam[&&]	X				X	X
Concomitant Medications (within 7 days)	X				X	X
Tumor Imaging (CTs and/or PET-CT**)		X				X
Assess toxic effects&					X[&&]	X
Research EGD, Biopsies, Photos (during phase II)	X			X		
Hematology (CBC, differential)	X		Weekly during therapy			X
Serum Chemistry Panel (sodium, potassium, , BUN, Cr, Calcium, Magnesium, ALT, AST, ALP, Total Bilirubin,creatinine phosphokinase (CPK)), Amylase, Lipase, glucose	X		Every 1 week during biochemotherapy			X
Serum Pregnancy Test@@	X					
Relapse-free survival and overall survival						X##

Note: All assessments should be performed within ± 3 days, unless otherwise specified. On treatment days, all assessments should be performed prior to dosing, unless otherwise specified.

* TNM clinical baseline staging will be determined by EGD, EUS, CTs, and PET-CT
** PET-CT will be obtained whenever possible, if not possible and CT is done if there is a clinical indication/question that can be answered by PET/CT this will be allowed in addition to CT.
& Adverse event reporting will be a continuous process
µ Surgical resection will be performed approximately 6-8 weeks from the end of biochemoradiation. If a delay in operation occurs, the reason(s) will be noted.
&&In phase IB, all patients will be seen in the clinic weekly, including having a physical exam, continuing for two weeks after the completion of biochemoradiation. Phase II patients will have a physical exam every 2 weeks and will also be monitored closely in the first two weeks after the completion of biochemoradiation but will not be necessarily seen in the clinic. Patients will be telephone-interviewed weekly and seen if necessary.
@@ In women with childbearing potential, serum pregnancy test will be performed within 14 days prior to the first dose of TALADEGIB. A serum pregnancy test will be administered every 4 weeks if their menstrual cycles are regular or every 2 weeks if their cycles are irregular while on biochemoradiation within the 24-hour period prior to the administration of TALADEGIB.
All patients will be followed for relapse-free survival and overall survival. Patients who continue to surgery will be followed as per Table 14. Other patients will be followed every 3 months starting 5-6 weeks after completion of biochemoradiation. Patient who no longer return to MD Anderson will be contacted by phone.

Table 14 Follow Up Tests and Observations After Surgery

Item	At 3 months	Every 3-6 months in Year 1	After year 1 Every 6 months x 4	At years 4 and 5
History and physical	Yes	Yes	Yes	Yes
CT scan or PET/CT*	Yes	Yes	Yes	Yes
EGD/EUS/Bx	Yes (with or without EUS)	No	As needed	No
CBC, diff	Yes	Yes	Yes	Yes
Serum chemistries, Na, K, Ca, Mg, BUN, Cr, AST, ALT, ALP, Total Bilirubin	Yes	Yes	Yes	Yes
PS	Yes	Yes	Yes	Yes

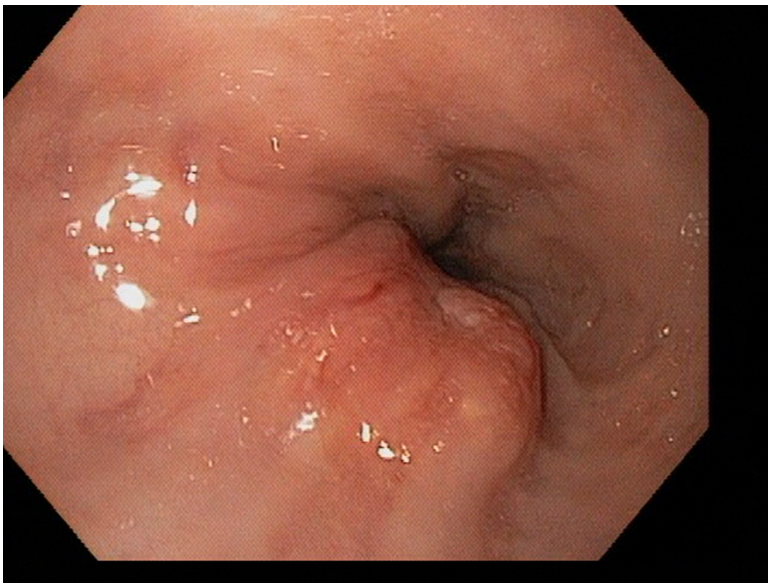
* PET can be replaced by CTs if it is not approved. If a dedicated CT Chest/Abdomen is needed at the 3 month visit in addition to the PET/CT it is allowed and left at the discretion of the treating physician.

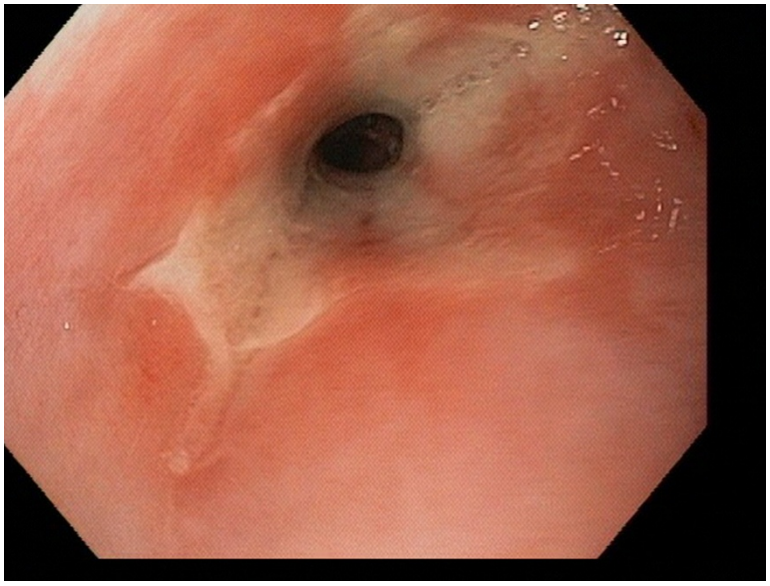
10. MEASUREMENT OF EFFECT

Although we propose to study adenocarcinoma of the esophagus and gastroesophageal junction in this protocol, the selected patient population will have only endoscopically assessable cancers (sometime, CTs or EUS can visualize lymph nodes). Endoscopic evaluation to assess objective response is not advised and standard criteria are lacking. Therefore, we will not use standard approach (RECIST) to assess response.

"Response" assessment is not the primary objective of this study. In the phase II part of the protocol, we will have an opportunity to endoscopically view the cancer in every patient. We plan to digitally record the size and shape of cancer serially (baseline, during therapy, and at the end of the recovery from biochemoradiation). No claims will be made from these observational studies. Only highly experienced gastroenterologists will participate in this study.

An example of digital photographs of an adenocarcinoma of the esophagus at baseline and





following therapy is provided above (top picture shows tumor before any therapy and bottom picture shows no tumor after chemoradiation).

The RECIST criteria do not apply.

10.1 Antitumor Effect – Solid Tumors

RECIST will not be applied to this study except in the event of progression during therapy.

10.1.1 Definitions

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

Evaluable for objective response. N/A

Evaluable Non-Target Disease Response. N/A

10.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions **Clinical** lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray **Lesions** on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which

greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions

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

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient

increase to qualify for PD, taking as reference the smallest sum diameters while on study.

10.1.4.2 Evaluation of Non-Target Lesions

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

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

10.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

10.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

10.1.6 Progression-Free Survival N/A

10.2 Other Response Parameters

The surgical specimen will graded as pathCR or <pathCR based on a number of criteria previously described.[15, 27]In short, pathCR will be designated if there is not histologic evidence of residual cancer cell in the resected specimen (includes lymph nodes). <pathCR will be designated if cancer cells are found in the resected specimen (includes nodes).

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

The primary objective of the phase IB part of the study is to assess the safety of TALADEGIB when used in combination with paclitaxel and carboplatin and radiation therapy. Up to 12 patients will be enrolled in this phase of the study. Initially, 6 patients will receive TALADEGIB at 400 mg/day for 38 days. If </= 1/6 patients develop dose-limiting toxicities (DLTs) (DLT period is from the start of TALADEGIB to 5 weeks after the completion of biochemoradiation) that are deemed to be associated with TALADEGIB, then the current dose/schedule combination of TALADEGIB (i.e., 400 mg/day for 38 days) is considered safe and will be used in phase II part of the study. Otherwise, if more than 1 DLT occurs in the first 6 patients, an additional cohort of 6 patients will receive TALADEGIB at 200 mg every day for 38 days. If this dose/schedule combination is established to be safe (i.e., with </= 1/6 DLTs), then this dose/schedule combination will be used in phase II part.

Phase II Design

The primary objective of the phase II part of the study is to assess the efficacy of TALADEGIB when used in combination with paclitaxel, carboplatin and radiation therapy. This phase will be conducted in 2 steps- first step will include treating 27 patients with TALADEGIB alone for 7 days (with a pre- and post- TALADEGIB endoscopic biopsy of EAC (7-10 days after the start of TALADEGIB but before starting chemoradiation); followed by administration of biochemoradiation (TALADEGIB, paclitaxel, carboplatin, radiation) and surgery. The second step will recruit additional 27 patients to undergo biochemoradiation and surgery.

The primary endpoint for the phase II part of the study is the pathologic complete response (pathCR) rate and a pathCR rate of at least 35% (>/= 40% is desirable) will be of interest. However, it is uncertain whether or not the additional 7-day treatment with TALADEGIB will increase the pathCR rate, relative to using biochemoradiotherapy only. Therefore, our primary goal here is not to compare between the two treatment steps, rather, we would like to estimate the pathCR rate in the TALADEGIB + biochemoradiotherapy step, using the

biochemoradiotherapy only step as a reference. With 27 patients per step and assuming that 10% of patients will not go to surgery, this will result in 24 patients per step that will undergo surgery. Assuming a pathCR rate of 35%, the half-width of the two-sided 95% confidence interval will be 0.191 given a sample size of 24.

For each treatment step, the Bayesian method of Thall and Simon[82] will be employed to perform interim safety monitoring. Denote θ_T as the probability of toxicity and assuming a non-informative beta(1,1) prior for θ_T, we will stop a step if, at any time during the study, we determine that there is more than 95% chance that the posterior probability of toxicity is more than 33%. Specifically, our interim monitoring rule for toxicity is: $Pr[\theta_T > 33\% \mid data] > 0.95$. The stopping boundaries corresponding to the above monitoring rule are to terminate a step if (# patients with toxicities) / (# patients evaluated) ≥ 4/5, 5/7, 6/9, 7/11, 8/13, 9/16, 10/18, 11/20, 12/23, 13/25.

The operating characteristics of this stopping rule are illustrated in Table 15.

Table 15. Operating characteristics of the Bayesian stopping rule for toxicity.

True toxicity probability	Early stopping probability	Achieved sample size 25^{th}, 50^{th}, 75^{th} percentiles		
0.1	0.002	27	27	27
0.2	0.025	27	27	27
0.3	0.136	27	27	27
0.4	0.402	12	27	27
0.5	0.740	6	12	27

All patients (up to 12 in phase I and 54 in phase II) will be enrolled from MDACC, at an accrual rate of 1-2 patients per month. The PI and his team monitor this single arm study. For phase I part, the safety data will be summarized using frequencies and percentages by adverse event (AE) category, grade and attributions. For phase II part, the pathCR rate in each of the treatment steps will be estimated, along with the 95% confidence interval.

Biomarker Studies: Several exploratory biomarkers will be studied. These will include potential biomarkers of primary and secondary resistance. For both the phase IB and phase II parts of the study, tissue samples will be collected and biomarker expression levels will be assessed at baseline and at the time of surgery. In addition, for phase II part of the study, patients will undergo a research endoscopy (RE) on day 8 (+/- 2 days) from the start of treatment. A linear mixed effect model will be fit to assess the change of biomarkers over time, which takes into consideration of the association among multiple marker observations from the same patient. The outcome variable will be biomarker expression level and the covariates will include time, treatment step and time by treatment interaction. The biomarker expression may be log-transformed prior to fit the model in order to satisfy the normality assumption. Also, we will fit a logistic regression model for the binary outcome of pathCR, using treatment step, baseline biomarker and the change of biomarker between baseline and at surgery as covariates. The interaction effect between time and biomarker (either baseline expression or the change of biomarker expression over time) will also be assessed when we fit the model. The biomarkers include expression of Shh, Ih, Gli1, and Gli2, Patch 2 and sFRP1 mRNA using real-time qPCR as well as refined and expand the pathway-associated markers

using gene expression signatures obtained from Aims 1 and 2**.** The refined gene signature list, (most likely ~100 genes) obtained from Aims 1 and 2, will be assayed using Low Density Gene Expression Custom Array (Micro Fluidic Card) which has the capacity to accommodate at most 100 genes. Custom tools have been developed at the Ohio State University Comprehensive Cancer Center by Dr. Kevin Coombes (a consultant for this SPORE) and will be used to analyze the data.

To study the correlation between biomarkers and the overall response (pathCR), we propose the following validation procedure. Specifically, we will integrate the observed biomarker measurements and patients' responses to form a combined data set. We will perform a leave-one-out cross validation (LOOCV) using this data set. In each iteration of the LOOCV, we will first remove one patient's response and biomarker measurements (denoted as the testing set), and use the measurements from the remaining patients (denoted as the training set) to train a logistic regression. Specifically, we will regress the binary responses from the training set on the corresponding biomarker measurements. Applying a step-wise variable selection in the logistic regression, we will select potentially predictive biomarkers for validation. This could be done in SAS, Splus, or other standard statistical software. We will estimate the regression coefficients after the final set of biomarkers is selected. Using the logit score based on the fitted logistic regression, we will develop a univariate prognostic index (a value between 0 and 1). We will compute the prognostic index for the patient in the testing set by plugging in their biomarker measurements. We will predict that the patient will respond to the treatment if the prognostic index is greater a predetermined threshold p_0 between 0 and 1. Going through the LOOCV with a different patient removed at a time, we will compute the sensitivity and specificity of the prediction model. By varying p_0 between 0 and 1 and, we will generate a receiver operating characteristic (ROC) curve. The area under the curve (AUC) will be computed to evaluate the performance of the prediction. An AUC value close to one indicates a good prediction while a value close to zero implies poor prediction.

11.2 Sample Size/Accrual Rate

We anticipate to enroll up to 66 patients in total from MD Anderson (up to 12 in phase 1b and 54 in phase II) at an enrollment rate of 1-2 patients per month.

11.3 Stratification Factors

None

11.4 Analysis of Secondary Endpoints

Stopping rule for toxicity are listed above and biomarker analysis is also discussed. Kaplan-Meier method will be used to estimate the probabilities of relapse-free survival and overall survival.

11.5 Reporting and Exclusions

11.5.1 Evaluation of Toxicity

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

11.5.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

APPENDIX A - PERFORMANCE STATUS CRITERIA

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

APPENDIX B - ACCEPTABLE FORMS OF CONTRACEPTION

Birth control pills
Depo-Provera®
Norplant®
IUD (intrauterine device)
Diaphragm with spermicide
Condom with spermicide

Women of childbearing potential and men are required to use two forms of acceptable contraception, including one barrier method, during their participation in the study and for 6 months following discontinuation of TALADEGIB.

The following are acceptable forms of contraception:

- Birth control pills
- Depo-Provera®
- Norplant®
- IUD (intrauterine device)
- Diaphragm with spermicide
- Condom with spermicide

APPENDIX C - PATIENT INFORMATION ON POSSIBLE DRUG INTERACTIONS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using the experimental agent. This form is addressed to the TALADEGIB *patient, but includes important information for others who care for this patient. A convenient wallet-sized information card is also included for the patient to clip out and retain at all times.*

TALADEGIB interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the counter remedy), or anything that you buy from the health food store or grocery store (herbal supplement).

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you**. These are the thing that you and they need to know:

- TALADEGIB and its major metabolite are CYP3A4 substrates. TALADEGIB also inhibits CYP3A4. These CYPs are enzymes in your liver. TALADEGIB must be used very carefully with other medicines that need certain liver enzymes to be effective or to be cleared from your system.
- The specific enzymes are CYP3A4.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inhibitors of CYP3A4."
- Your regular prescribers should look at this web site http://medicine.iupui.edu/clinpharm/ddis/table.asp to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and printed on the ingredient list. Find the generic name (your pharmacist can help) and look at the table on the back of this page. Be careful:
    - If you take acetaminophen regularly. You should not take more than 4 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - If you take herbal medicine regularly. You should not take St. John's wort while you are taking TALADEGIB.

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is _____

and he or she can be contacted at 713-792-2828.

_____

INFORMATION ON POSSIBLE DRUG INTERACTIONS	
You are enrolled on a clinical trial using the experimental agent **TALADEGIB.** . **TALADEGIB** interacts with drugs that are processed by your liver. Because of this, it is very important to: ➢ Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. ➢ Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial. ➢ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.	**TALADEGIB** interacts with specific liver enzymes named **CYP3A4/,** and must be used very carefully with other medicines that interact with these enzymes. ➢ Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inhibitors of **CYP_____**." ➢ Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/table.aspx for a list of drugs to avoid, or contact your study doctor. ➢ Your study doctor's name is **_____** and can be contacted at

APPENDIX D - PROHIBITED MEDICATIONS

CYP3A4 INHIBITORS List of medications prohibited in this study (cyp 450)

Strong Inhibitors of CYP3A
boceprevir
clarithromycin
conivaptan
grapefruit juice[a]
indinavir
itraconazole
ketoconazole
lopinavir/ritonavir
mibefradil[b]
nefazodone
nelfinavir
posaconazole
ritonavir
saquinavir
telaprevir
telithromycin
voriconazole

Source: US Food and Drug Administration.

[a] The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Studies have shown that it can be classified as a "strong CYP3A inhibitor" when a certain preparation was used (e.g., high dose, double strength) or as a "moderate CYP3A inhibitor" when another preparation was used (e.g., low dose, single strength).

[b] Withdrawn from the United States market because of safety reasons.

BIBLIOGRAPHY

1. Parkin, D.M., et al., *Global cancer statistics, 2002.* CA Cancer J Clin, 2005. **55**(2): p. 74-108.
2. *Oesophageal (gullet) cancer*. 2008 [cited 2008 October 14, 2008]; Incidence statistics]. Available from: http://info.cancerresearchuk.org/cancerstats/types/oesophagus/incidence/.
3. Brown, L.M., S.S. Devesa, and W.H. Chow, *Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age.* J Natl Cancer Inst, 2008. **100**(16): p. 1184-7.
4. Pohl, H. and H.G. Welch, *The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence.* J Natl Cancer Inst, 2005. **97**(2): p. 142-6.
5. Brown, L.M., et al., *Adenocarcinoma of the esophagus: role of obesity and diet.* J Natl Cancer Inst, 1995. **87**(2): p. 104-9.
6. Rice, T.W., et al., *Worldwide esophageal cancer collaboration.* Dis Esophagus, 2009. **22**(1): p. 1-8.
7. Tepper, J., et al., *Phase III trial of trimodality therapy with cisplatin, fluorouracil, radiotherapy, and surgery compared with surgery alone for esophageal cancer: CALGB 9781.* J Clin Oncol, 2008. **26**(7): p. 1086-92.
8. Walsh, T.N., et al., *A comparison of multimodal therapy and surgery for esophageal adenocarcinoma.* N Engl J Med, 1996. **335**(7): p. 462-7.
9. Bosset, J.F., et al., *Chemoradiotherapy followed by surgery compared with surgery alone in squamous-cell cancer of the esophagus.* N Engl J Med, 1997. **337**(3): p. 161-7.
10. Urba, S.G., et al., *Randomized trial of preoperative chemoradiation versus surgery alone in patients with locoregional esophageal carcinoma.* J Clin Oncol, 2001. **19**(2): p. 305-13.
11. Suntharalingam, M., et al., *The national practice for patients receiving radiation therapy for carcinoma of the esophagus: results of the 1996-1999 Patterns of Care Study.* Int J Radiat Oncol Biol Phys, 2003. **56**(4): p. 981-7.
12. Suntharalingam, M., et al., *Outcome results of the 1996-1999 patterns of care survey of the national practice for patients receiving radiation therapy for carcinoma of the esophagus.* J Clin Oncol, 2005. **23**(10): p. 2325-31.
13. Ajani, J.A., et al., *Phase II randomized trial of two nonoperative regimens of induction chemotherapy followed by chemoradiation in patients with localized carcinoma of the esophagus: RTOG 0113.* J Clin Oncol, 2008. **26**(28): p. 4551-6.
14. Kelsen, D.P., et al., *Long-term results of RTOG trial 8911 (USA Intergroup 113): a random assignment trial comparison of chemotherapy followed by surgery compared with surgery alone for esophageal cancer.* J Clin Oncol, 2007. **25**(24): p. 3719-25.
15. Chirieac, L.R., et al., *Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation.* Cancer, 2005. **103**(7): p. 1347-55.
16. Javeri, H., et al., *Influence of induction chemotherapy and class of cytotoxics on pathologic response and survival after preoperative chemoradiation in patients with carcinoma of the esophagus.* Cancer, 2008. **113**(6): p. 1302-8.

17. Rizk, N.P., et al., *American Joint Committee on Cancer staging system does not accurately predict survival in patients receiving multimodality therapy for esophageal adenocarcinoma.* J Clin Oncol, 2007. **25**(5): p. 507-12.
18. Bartels, H., H.J. Stein, and J.R. Siewert, *Risk analysis in esophageal surgery.* Recent Results Cancer Res, 2000. **155**: p. 89-96.
19. Izzo, J.G., et al., *Clinical biology of esophageal adenocarcinoma after surgery is influenced by nuclear factor-kappaB expression.* Cancer Epidemiol Biomarkers Prev, 2007. **16**(6): p. 1200-5.
20. Siewert, J.R., *[Esophageal carcinoma].* Chirurg, 2007. **78**(5): p. 475-84; quiz 485.
21. Izzo, J.G., et al., *Therapy-induced expression of NF-kappaB portends poor prognosis in patients with localized esophageal cancer undergoing preoperative chemoradiation.* Dis Esophagus, 2008.
22. Jiang, Y., et al., *Upper gastrointestinal tumors: current status and future perspectives.* Expert Rev Anticancer Ther, 2008. **8**(6): p. 975-91.
23. Siersema, P.D. and R. van Hillegersberg, *Treatment of locally advanced esophageal cancer with surgery and chemoradiation.* Curr Opin Gastroenterol, 2008. **24**(4): p. 535-40.
24. Rohatgi, P., et al., *Characterization of pathologic complete response after preoperative chemoradiotherapy in carcinoma of the esophagus and outcome after pathologic complete response.* Cancer, 2005. **104**(11): p. 2365-72.
25. Berger, A.C., et al., *Complete response to neoadjuvant chemoradiotherapy in esophageal carcinoma is associated with significantly improved survival.* J Clin Oncol, 2005. **23**(19): p. 4330-7.
26. Donahue, J.M., et al., *Complete pathologic response after neoadjuvant chemoradiotherapy for esophageal cancer is associated with enhanced survival.* Ann Thorac Surg, 2009. **87**(2): p. 392-8; discussion 398-9.
27. Wu, T.T., et al., *Excellent interobserver agreement on grading the extent of residual carcinoma after preoperative chemoradiation in esophageal and esophagogastric junction carcinoma: a reliable predictor for patient outcome.* Am J Surg Pathol, 2007. **31**(1): p. 58-64.
28. Rohatgi, P.R., et al., *Failure patterns correlate with the proportion of residual carcinoma after preoperative chemoradiotherapy for carcinoma of the esophagus.* Cancer, 2005. **104**(7): p. 1349-55.
29. Luthra, R., et al., *Gene expression profiling of localized esophageal carcinomas: association with pathologic response to preoperative chemoradiation.* J Clin Oncol, 2006. **24**(2): p. 259-67.
30. Wang, Y., et al., *The crosstalk of mTOR/S6K1 and Hedgehog pathways.* Cancer Cell, 2012. **21**(3): p. 374-87.
31. Yang, L., et al., *Hedgehog signaling activation in the development of squamous cell carcinoma and adenocarcinoma of esophagus.* Int J Biochem Mol Biol, 2012. **3**(1): p. 46-57.
32. Sims-Mourtada, J., et al., *Hedgehog: an attribute to tumor regrowth after chemoradiotherapy and a target to improve radiation response.* Clin Cancer Res, 2006. **12**(21): p. 6565-72.
33. Sims-Mourtada, J., et al., *Sonic Hedgehog promotes multiple drug resistance by regulation of drug transport.* Oncogene, 2007. **26**(38): p. 5674-9.

34. Izzo, J.G., et al., *Association of activated transcription factor nuclear factor kappab with chemoradiation resistance and poor outcome in esophageal carcinoma.* J Clin Oncol, 2006. **24**(5): p. 748-54.
35. Izzo, J., et al., *Correlation of pre-treatment NF-kB status with chemoradiation resistance and metastatic progression irrespective of the type of chemotherapy used with radiation for esophageal carcinoma.* Mol. Cancer Therapeutics, 2006. **5**: p. 2844-2850.
36. Sims-Mourtada, J., et al., *Sonic Hedgehog promotes multiple drug resistance by regulation of drug transport.* Oncogene, 2007. **26**: p. 5674-5679.
37. Ingham, P.W. and A.P. McMahon, *Hedgehog signaling in animal development: paradigms and principles.* Genes Dev, 2001. **15**(23): p. 3059-87.
38. Evangelista, M., H. Tian, and F.J. de Sauvage, *The hedgehog signaling pathway in cancer.* Clin Cancer Res, 2006. **12**(20 Pt 1): p. 5924-8.
39. Chari, N.S. and T.J. McDonnell, *The sonic hedgehog signaling network in development and neoplasia.* Adv Anat Pathol, 2007. **14**(5): p. 344-52.
40. Romer, J. and T. Curran, *Targeting medulloblastoma: small-molecule inhibitors of the Sonic Hedgehog pathway as potential cancer therapeutics.* Cancer Res, 2005. **65**(12): p. 4975-8.
41. Lupi, O., *Correlations between the Sonic Hedgehog pathway and basal cell carcinoma.* Int J Dermatol, 2007. **46**(11): p. 1113-7.
42. Berman, D.M., et al., *Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours.* Nature, 2003. **425**(6960): p. 846-51.
43. Thayer, S.P., et al., *Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis.* Nature, 2003. **425**(6960): p. 851-6.
44. Karhadkar, S.S., et al., *Hedgehog signalling in prostate regeneration, neoplasia and metastasis.* Nature, 2004. **431**(7009): p. 707-12.
45. Rubin, L.L. and F.J. de Sauvage, *Targeting the Hedgehog pathway in cancer.* Nat Rev Drug Discov, 2006. **5**(12): p. 1026-33.
46. Cooper, J.S., et al., *Chemoradiotherapy of locally advanced esophageal cancer: long-term follow-up of a prospective randomized trial (RTOG 85-01). Radiation Therapy Oncology Group.* Jama, 1999. **281**(17): p. 1623-7.
47. Ajani, J.A., et al., *Esophageal cancer.* J Natl Compr Canc Netw, 2008. **6**(9): p. 818-49.
48. Kelsey, C.R., et al., *Paclitaxel-based chemoradiotherapy in the treatment of patients with operable esophageal cancer.* Int J Radiat Oncol Biol Phys, 2007. **69**(3): p. 770-6.
49. Bader, F.G., et al., *Paclitaxel in the neoadjuvant treatment for adeno carcinoma of the distal esophagus (AEG I). A comparison of two phase II trials with long-term follow-up.* Onkologie, 2008. **31**(7): p. 366-72.
50. Ajani, J.A., et al., *Paclitaxel-based chemoradiotherapy in localized gastric carcinoma: degree of pathologic response and not clinical parameters dictated patient outcome.* J Clin Oncol, 2005. **23**(6): p. 1237-44.
51. Ajani, J.A., et al., *Multi-institutional trial of preoperative chemoradiotherapy in patients with potentially resectable gastric carcinoma.* J Clin Oncol, 2004. **22**(14): p. 2774-80.
52. Ajani, J.A., et al., *Phase II trial of preoperative chemoradiation in patients with localized gastric adenocarcinoma (RTOG 9904): quality of combined modality therapy and pathologic response.* J Clin Oncol, 2006. **24**(24): p. 3953-8.
53. van Hagen, P., et al., *Preoperative chemoradiotherapy for esophageal or junctional cancer.* N Engl J Med, 2012. **366**(22): p. 2074-84.

54. Lum, L. and P.A. Beachy, *The Hedgehog response network: sensors, switches, and routers.* Science, 2004. **304**(5678): p. 1755-9.
55. Hooper, J.E. and M.P. Scott, *Communicating with Hedgehogs.* Nat Rev Mol Cell Biol, 2005. **6**(4): p. 306-17.
56. Kasper, M., et al., *GLI transcription factors: mediators of oncogenic Hedgehog signalling.* Eur J Cancer, 2006. **42**(4): p. 437-45.
57. Wetmore, C., *Sonic hedgehog in normal and neoplastic proliferation: insight gained from human tumors and animal models.* Curr Opin Genet Dev, 2003. **13**(1): p. 34-42.
58. Murone, M., A. Rosenthal, and F.J. de Sauvage, *Sonic hedgehog signaling by the patched-smoothened receptor complex.* Curr Biol, 1999. **9**(2): p. 76-84.
59. Neumann, C.J., *Hedgehogs as negative regulators of the cell cycle.* Cell Cycle, 2005. **4**(9): p. 1139-40.
60. Colnot, C., et al., *Indian hedgehog synchronizes skeletal angiogenesis and perichondrial maturation with cartilage development.* Development, 2005. **132**(5): p. 1057-67.
61. Athar, M., et al., *Hedgehog signalling in skin development and cancer.* Exp Dermatol, 2006. **15**(9): p. 667-77.
62. Dellovade, T., et al., *The Hedgehog Pathway and Neurological Disorders.* Annu Rev Neurosci, 2006.
63. Blanpain, C. and E. Fuchs, *Epidermal Stem Cells of the Skin.* Annu Rev Cell Dev Biol., 2006. **22**: p. 35.
64. Kinzler, K.W., et al., *Identification of an amplified, highly expressed gene in a human glioma.* Science, 1987. **236**(4797): p. 70-3.
65. Pasca di Magliano, M. and M. Hebrok, *Hedgehog signalling in cancer formation and maintenance.* Nat Rev Cancer, 2003. **3**(12): p. 903-11.
66. Lees, C., et al., *The hedgehog signalling pathway in the gastrointestinal tract: implications for development, homeostasis, and disease.* Gastroenterology, 2005. **129**(5): p. 1696-710.
67. Eichberger, T., Sander, V., Schnider, H., et al. , *Overlapping and distinct transcriptional regulator properties of the GLI-1 and GLI-2 oncogenes.* 2006, 2006. **87**: p. 616-632.
68. Palma, V. and A. Ruiz i Altaba, *Hedgehog-GLI signaling regulates the behavior of cells with stem cell properties in the developing neocortex.* Development, 2004. **131**(2): p. 337-45.
69. Beachy, P.A., S.S. Karhadkar, and D.M. Berman, *Tissue repair and stem cell renewal in carcinogenesis.* Nature, 2004. **432**(7015): p. 324-31.
70. Beachy, P.A., S.S. Karhadkar, and D.M. Berman, *Mending and malignancy.* Nature, 2004. **431**(7007): p. 402.
71. Zhao, C., et al., *Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia.* Nature, 2009. **458**(7239): p. 776-9.
72. Lee, C.J., J. Dosch, and D.M. Simeone, *Pancreatic cancer stem cells.* J Clin Oncol, 2008. **26**(17): p. 2806-12.
73. Lamm, M.L., et al., *Sonic hedgehog activates mesenchymal Gli1 expression during prostate ductal bud formation.* Dev Biol, 2002. **249**(2): p. 349-66.
74. Zhang, J., et al., *Lack of demonstrable autocrine hedgehog signaling in human prostate cancer cell lines.* J Urol, 2007. **177**(3): p. 1179-85.
75. Yauch, R.L., et al., *A paracrine requirement for hedgehog signalling in cancer.* Nature, 2008. **455**(7211): p. 406-10.

76. Davis, D.W., et al., *Automated quantification of apoptosis after neoadjuvant chemotherapy for breast cancer: early assessment predicts clinical response.* Clin Cancer Res, 2003. **9**(3): p. 955-60.
77. Ajani, J.A., et al., *Molecular Biomarkers Correlate with Disease-Free Survival in Patients with Anal Canal Carcinoma Treated with Chemoradiation.* Dig Dis Sci, 2009.
78. Izzo, J.G., et al., *Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma.* J Clin Oncol, 2007. **25**(6): p. 698-707.
79. Izzo, J., et al., *Cyclin D1 genotype, response to biochemoprevention, and progression rate to upper aerodigestive tract cancer.* J Natl Cancer Inst, 2003. **95**(3): p. 198-205.
80. Izzo, J.G., et al., *Pretherapy nuclear factor-kappaB status, chemoradiation resistance, and metastatic progression in esophageal carcinoma.* Mol Cancer Ther, 2006. **5**(11): p. 2844-50.
81. Izzo, J.G., et al., *Dysregulated cyclin D1 expression early in head and neck tumorigenesis: in vivo evidence for an association with subsequent gene amplification.* Oncogene, 1998. **17**(18): p. 2313-22.
82. Thall, P.F. and R. Simon, *A Bayesian approach to establishing sample size and monitoring criteria for phase II clinical trials.* Control Clin Trials, 1994. **15**(6): p. 463-81.