Version date: 01-May-2005

Tarceva Investigator Sponsored Trial PROTOCOL TEMPLATE

TITLE:	**A Phase II Exploratory Study of Pre-Operative Treatment with Erlotinib (Tarceva) in Muscle Invasive or Recurrent Transitional Cell Carcinoma Requiring Cystectomy**
PROTOCOL NUMBER:	OSI4222
STUDY DRUG:	Tarceva™ (erlotinib; OSI-774)
PRINCIPAL INVESTIGATOR	Arlene Siefker-Radtke, M. D.

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1. BACKGROUND

There is growing evidence supporting neoadjuvant therapy for transitional cell carcinomas confined to the bladder.[1] However, for the appropriately selected group of patients with tumor invading the muscle wall (i.e. clinical T2 tumors), the evidence is not so clear. Though many patients may be cured by surgical resection alone, there is a subset of these patients who relapse and develop incurable metastatic disease. Therefore, while one may not want to put these patients through the toxicity of systemic multi-agent chemotherapy, there is certainly the feeling that something further must be done. Since, at the current time, these patients often undergo radical cystectomy where the entire bladder, prostate and regional lymph nodes are removed, this group of untreated patients with intact bladders provides an ideal opportunity to explore new agents and collect tissue specimens to measure candidate prospective markers of response, receptor phosphorylation, and other downstream targets of biological agents.

Among the numerous biological targets implicated in transitional cell carcinoma progression, inhibitors of the epidermal growth factor receptor (EGF-R) remain extremely attractive. The receptor for EGF-R is over-expressed by more than 75% of all human TCC, with the level of expression correlating directly with tumor grade, stage, and survival.[2] Numerous studies have demonstrated that clinically relevant inhibitors of the EGF-R, strongly inhibit the growth of human bladder tumor xenografts.[3,4] Thus, there is compelling rationale to target these pathways in this patient population. However, EGFR inhibitors have not displayed consistent activity in clinical trials performed in other disease sites, which has dampened clinical enthusiasm for aggressively developing them further. Studies performed within the context of non-small cell lung cancer demonstrated that clinical responses were linked to activating mutations within the EGFR tyrosine kinase domain, suggesting that a better understanding of the biological effects of EGFR inhibitors on tumor cells will help to identify tumors that will respond (biologically or clinically) to therapy.

Although none of the urothelial carcinoma cell lines nor any of 75 primary tumors we evaluated contained activating EGFR kinase domain mutations or expressed the truncated form of the EGFR (EGFRvIII) that mediates ligand-independent signaling and promotes tumor growth in other model systems, we found that EGFR inhibitors (gefitinib or cetuximab) blocked cell cycle progression in 8/20 human bladder cancer cell lines. Through a large collaborative effort in our Bladder SPORE program, with a Core Project studying EGF-R inhibition in urothelial cancer, we have identified molecular mechanisms that appear to correlate with or mediate these effects.

Consistent with emerging evidence from other solid tumors, is the so-called “epithelial to mesenchymal transition” (EMT) which correlates with resistance

to EGFR antagonist-induced growth inhibition. Specifically, the sensitivity to EGFR inhibition in urothelial carcinoma as defined by cell cycle arrest was proportional to the pre-treatment expression of E-cadherin and HER4, and inversely proportional to the expression of PDGFR-β, a mesenchymal growth factor receptor. Moreover, the response to therapy could be predicted 'early' in the course of treatment by quantifying changes in the expression of proliferation markers, including Ki-67/PCNA, p27, Cyclin D1, and GSK-3-β. Thus, we have designed a proof-of-principle clinical trial to provide information to prospectively identify tumors likely to respond to this form of therapy. Knowledge gained from this trial may have substantial impact in bladder cancer, including the setting of secondary chemoprevention, or in future combinations with other agents.

1.1 EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION AND SIGNIFICANCE IN CANCER

The control of cell growth is mediated by a complex network of signaling pathways responsive to external influences, such as growth factors, as well as to internal controls and checks. Epidermal growth factor (EGF) was one of the first growth factors to be described. It was shown to be mitogenic, an effect mediated by the binding of EGF (or other ligands) to the cell surface EGF receptor (EGFR), stimulating autophosphorylation of the intracellular tyrosine kinase domain of the receptor. Subsequent investigations revealed EGFR to be one of a family of closely related receptors that includes EGFR (HER1), HER2, HER3, and HER4.

EGFR and other HER family members are considered to be important in the development, progression, and aggressive behavior of human epithelial malignancies and to be relevant therapeutic targets. A number of human malignancies are associated with aberrant or over-expression of EGFR (Salomon et al. 1995). Stimulation of tumor cells via the EGFR is important for both tumor growth and tumor survival in vivo. Over-expression of EGFR in certain human tumors, including non–small cell lung carcinoma (NSCLC), has been correlated with both chemo-resistance and poor prognosis (Rusch et al. 1996, 1997; Davies and Chamberlin 1996; Veale et al. 1987, 1993; Sekine et al. 1998; Pfeiffer et al. 1996; Cerny et al. 1986; IMPATH Inc 1998–1999; Reissmann et al. 1999; Fujino 1996; Fontanini et al. 1995; Lei et al. 1999). Inhibitors of EGFR tyrosine kinase activity have been in development for a number of years, and although earlier compounds lacked specificity and potency, newer compounds have proven active in nonclinical and clinical studies.

Tarceva (previously known as OSI-774) is an orally active, potent, selective inhibitor of the EGFR tyrosine kinase. Early clinical data with Tarceva

indicate that the compound is generally safe and well tolerated at doses that provide the targeted effective concentration based on nonclinical experiments. A recently completed, randomized, double-blind, placebo-controlled trial has shown that Tarceva as a single agent significantly improves the survival of patients with incurable Stage IIIb/IV NSCLC who have failed standard therapy for advanced or metastatic disease (Shepherd et al; Proceedings of ASCO 2004; Abst 7022).

1.2 TARCEVA AS AN EGFR TYROSINE KINASE INHIBITOR

Tarceva is an EGFR tyrosine kinase inhibitor that has been investigated in several Phase III studies. An overview of relevant nonclinical and clinical information is presented below; complete details are available in the Tarceva™ Investigator Brochure.

1.2.1 Non-clinical Data

a. Pharmacology

Tarceva, a quinazoline, directly and reversibly inhibits the human EGFR tyrosine kinase with an IC_{50} of 2 nM (0.79 ng/mL) in an in vitro enzyme assay and reduces EGFR autophosphorylation in intact tumor cells with an IC_{50} of 20 nM (7.9 ng/mL). This potent inhibition is selective for the EGFR tyrosine kinase both in assays assessing the effects of Tarceva on a variety of other isolated tyrosine kinases and in cellular bioassays designed to isolate this functional pathway. Tarceva is designed to inhibit EGF-dependent proliferation of cells at submicromolar concentrations and blocks cell cycle progression in the G1 phase.

Data on drug exposure and anti-tumor responses in human tumor xenograft models (HN5 and A431) were analyzed in order to estimate the plasma concentration of erlotinib associated with anti-tumor activity. Based on these efficacy models, the minimum steady-state plasma concentration targeted for clinical activity in humans is projected to be 500 ng/mL.

b. Toxicology

Toxicology studies have been performed in mice, rats (up to 6 months), dogs (up to 1 year), and monkeys (1 week). Treatment-related effects observed in at least one species or study included effects on the cornea (atrophy, ulceration), skin (follicular degeneration and inflammation, redness, and alopecia), ovary (atrophy), liver (necrosis), kidney (papillary necrosis and tubular dilatation), lacrimal glands (atrophy), salivary glands (atrophy), mandibular lymph nodes (inflammation), spleen (hematopoiesis), gastrointestinal tract (delayed gastric emptying and diarrhea), and embryo-fetal toxicity. Red blood cell parameters were decreased, and white blood cells (primarily neutrophils) were increased. There were treatment-related

increases in ALT, AST, triglyceride and bilirubin and decreases in albumin; increases in bilirubin were likely caused by a treatment-related impairment of bilirubin metabolism.

1.2.2 Clinical Experience with Tarceva

a. Dose Selection for Single-Agent Trials of Tarceva

Phase I trials of Tarceva explored both schedule and dose to evaluate the safety, tolerability, and pharmacokinetic profile of the compound given as a single agent. A number of pharmacokinetic trials in healthy subjects have been conducted, along with three classic Phase I trials in patients with advanced cancer. The single-agent maximum tolerated dose (MTD) was estimated to be 150 mg administered once daily.

The primary toxicities of single-agent Tarceva consisted of rash (dermatosis), diarrhea, nausea, fatigue, stomatitis, vomiting, and headache. When given daily, dose-limiting toxicity (diarrhea) was observed at 200 mg/day. At 150 mg/day, diarrhea was manageable with the addition of loperamide therapy; this dose was considered the maximal tolerated dose.

Rash (variously referred to as dermatitis, acneiform rash, or maculopapular rash) has been variable in onset, duration, and severity, but typically appears on the face, neck, scalp, chest, and back starting after ~1 week of treatment. The mechanistic basis of the rash remains uncertain; histopathologic examination of biopsies of the rash demonstrated inflammatory cell infiltrate and mild epidermal hyperproliferation. In some cases, the rash gradually improved despite continued dosing and, in general, resolved without sequelae following Tarceva discontinuation. The rash did not result in study discontinuation in patients with cancer in the Phase I trials. Laboratory abnormalities observed infrequently with single-agent Tarceva involved primarily liver function tests, including elevation of ALT, AST, and/or bilirubin.

Selection of the 150 mg/day dose of Tarceva for subsequent single-agent studies was based on pharmacokinetic parameters, as well as the safety and tolerability profile of this dose in Phase I trials in heavily pretreated patients with advanced cancer. Drug levels seen in patients with cancer receiving the 150 mg/day dose were consistently above the average plasma concentration of 500 ng/mL targeted for clinical efficacy.

b. Pharmacokinetics

Oral Tarceva is well absorbed and has an extended absorption phase, with mean peak plasma levels occurring at 3 hours after oral dosing of 150 mg/dL at steady state. A study in healthy subjects provided an estimate of bioavailability of 59% (95% CI: 55%, 63%). The time to reach steady-state plasma concentration was ~5 days. The accumulation ratio with daily dosing

of Tarceva was estimated to be 2.0. From a population pharmacokinetic analysis of 708 patients, the median trough concentration (C_{min}) 24 hours following the previous dose was 1041 ($\pm$697) ng/mL. Median AUC achieved during the dosing interval at steady state was 19,801 ng • hr/mL. Exposure after an oral dose is increased by food.

There is extensive binding of Tarceva and metabolites to both serum albumin and AAG (alpha-1-acid glycoprotein), with total plasma protein binding for Tarceva and OSI-420 of ~95% and 91%, respectively. Tarceva is extensively metabolized in the liver by the hepatic cytochromes in humans–primarily by CYP3A4 and to a lesser extent by CYP1A2. The primary metabolite of Tarceva, OSI-420, has potency comparable to that of erlotinib, but is present at levels that are $<$10% of erlotinib levels. Tarceva is excreted predominantly via the feces ($>$90%). The elimination half-life after a 150-mg oral dose is ~30 hours. In population-based data analyses, no relationships were identified between predicted steady-state trough concentration and patient age, body weight, sex, ethnicity, or creatinine clearance.

c. Phase II and III Trials in Patients with Advanced Cancer

Multiple Phase II trials evaluating the safety, tolerability, and antitumor activity of Tarceva have been conducted in patients with advanced, refractory malignancies including cancer of the head and neck, lung, aerodigestive tract, ovary, breast, central nervous system (glioma), and others. Tarceva has been evaluated both as a single agent and administered concurrently with conventional chemotherapy agents using various doses and schedules.

Evidence of activity has been observed in squamous cell carcinoma of the head and neck, ovarian, breast and pancreatic carcinoma, non–small cell lung cancer (NSCLC), and glioblastoma multiforme (GBM). Patients received 150 mg/day of Tarceva in all of these studies except the GBM study where dose escalation was allowed until limited by rash and where a higher starting dose was tested in subjects receiving concomitant enzyme inducing anti-epileptic drugs. Dose reduction was allowed in all studies in the case of intolerance. Diarrhea was treated with loperamide therapy and/or dose reduction. Rash was treated with a variety of agents, including oral and topical antibiotics, corticosteroids, and other agents.

Patients receiving Tarceva in combination with various chemotherapy agents have generally experienced the same type of adverse events (AEs) as with either agent alone.

The first randomized placebo controlled trial to demonstrate a survival advantage for an EGFR inhibitor was the Phase III study, BR21. This international trial, conducted by the National Cancer Institute of Canada Clinical Trial Group (NCIC CTG), included 731 patients with incurable Stage

IIIb/IV NSCLC who have failed standard therapy for advanced or metastatic disease. Patients randomized in a 2:1 ratio to single-agent Tarceva 150 mg/day obtained a 42.5% improvement in median survival over placebo, from 4.7 to 6.7 months. The one-year survival increased significantly (from 22% to 31%) as did the median and 6 month PFS, response rate, and the time to deterioration of tumor related symptoms of pain, cough, and dyspnea. (Shepherd 2004).

In BR-21, of the 727 patients evaluable for safety (485 Tarceva, 242 placebo), the most common AEs in the Tarceva arm were rash (75% Tarceva, 17% placebo), diarrhea (54% Tarceva, 18% placebo) and stomatitis (17% Tarceva, 18% placebo) events. The majority of these events were mild to moderate in severity. The incidence of ILD reported was the same in the placebo and Tarceva groups at 0.8% in each arm.

Two large, Phase III, randomized studies in first-line NSCLC patients evaluated Tarceva in combination with platinum-based two-drug combination chemotherapy. A total of 1079 previously untreated patients received carboplatin/paclitaxel with either Tarceva or placebo in the TRIBUTE trial (OSI2298g) conducted in the United States. An additional 1172 patients received cisplatin/gemcitabine plus either Tarceva or placebo in the TALENT trial (BO16411) conducted in 27 countries in Europe and other ex-U.S. locations. Neither study met its primary endpoint of improved overall survival or a secondary endpoint of improved time to disease progression or overall response rate. Overall, the number of adverse events and serious adverse events were well balanced between the two arms of each study, with two exceptions. As expected, rash and diarrhea occurred more frequently in the Tarceva arms. In the TRIBUTE study, more serious adverse events resulting in death were seen in the Tarceva arm compared with the placebo arm (53 vs. 27). Most of the apparent imbalance was due to events reported as pneumonia or progression of underlying cancer. (Gatzemeier U. et al.- Talent trial - ASCO 2004 - Abstract 7010 and Herbst R. et al. – Tribute trial - ASCO 2004 - Abstract 7011).

d. Patients with Hepatic or Renal impairment

The influence of hepatic metastases and/or hepatic dysfunction on the pharmacokinetics of Tarceva is not yet known. However, Tarceva is cleared predominately by the liver, and caution should be used when administering Tarceva to patients with hepatic dysfunction. Tarceva is also a strong inhibitor of the UDP-glucuronosyltransferase UGT1A1 enzyme responsible for the glucuronidation of bilirubin. Hyperbilirubinemia appears most often to be a side effect related to genetic polymorphisms of UGT1A1. Rare cases of hepatic failure (including fatalities) have been reported during the postmarketing use of Tarceva. Confounding factors for severe hepatic

dysfunction have included pre-existing liver disease such as cirrhosis, viral hepatitis, hepatocellular carcinoma, hepatic metastases, or concomitant treatment with potentially hepatotoxic drugs.

Rare cases of myocardial infarction (including fatalities) have been reported during the postmarketing use of Tarceva.

No clinical studies have been conducted in patients with compromised renal function since Tarceva and its metabolites are not significantly excreted by the kidneys.

1.3 STUDY RATIONALE

The receptor for EGF-R is over-expressed in more than 75% of all human transitional cell tumors, with the level of expression correlating directly with tumor grade, stage, and survival. In our pre-clinical models, clinically relevant inhibitors of the EGF-R strongly inhibit the growth of tumor cells in xenograft models. While EGF-R mutational status has been predictive of response within the context of non-small cell lung cancer, we have not found these mutations to present in our cell lines nor in 75 primary tumors that were evaluated for activating EGFR kinase domain mutations or the truncated form of the EGF-R (EGFRvIII). Despite these findings, we have observed that EGFR inhibitors blocked cell cycle progression in 8/20 human bladder cancer cell lines. Through a large collaborative effort in our Bladder SPORE program we have identified molecular mechanisms that appear to correlate with or mediate these effects.

While the primary endpoint will be focused on clinical response, it is clear that that a strong inducement for this study is the proposed biologic endpoints. With our current model, we have the opportunity to study novel agents in the preoperative setting with the ability to collect tissue both prior to therapy (with tissue collected on routine cystoscopy), and at cystectomy. Ultimately, we hope to use the knowledge gained from this descriptive study to identify appropriate patients for EGFR-directed or other selectively targeted therapies.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

To estimate the response rate (ie: pT0 rate) of patients with urothelial cancer treated with erlotinib prior to cystectomy. In this context, response will be defined as the absence of residual cancer in the resected specimen.

2.2 SECONDARY OBJECTIVE(S)

Clinical Objectives:

1. To estimate the 4-year disease-free survival of patients with urothelial cancer treated with erlotinib prior to cystectomy.

Biologic Objectives:

1. To measure EMT markers (E-cadherin, HER4, PDGFR-beta, vimentin, fibronectin) in pre- and post-treatment biopsies and correlate expression patterns with the biological responses measured below.
2. To quantify target inhibition, antiproliferation (KI-67), and apoptosis (TUNEL) in biopsies obtained from patients before, during, and after therapy.
3. Interrogate membrane (phosphorylated EGFR) and downstream receptor signaling pathways (ERKs, AKT/mTOR, GSK-3beta) to provide further insight into whether or not a given tumor displays a biological response.
4. To correlate the changes in Ki-67 expression with changes in angiogenesis and angiogenesis related gene expression utilizing fluorescent tissue staining techniques that we have developed in the laboratory (such as two-color TUNEL, phosphor-receptor, and microvessel density.)
5. To profile mRNA expression in pre- and post-treatment biopsies using Affymetrix arrays and correlate the changes observed with EMT, growth arrest, and apoptosis.
6. To quantify EGFR copy number and correlate with changes observed with EMT, growth arrest, and apoptosis.

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

Population:

Patients must have invasive transitional cell carcinoma, and be candidates for cystectomy. Patients with transitional cell tumors of the upper tract and urethra tumors may also be eligible. Patients with high-risk features including micropapillary features, small cell carcinoma, 3-D mass on EUA, and lymphovascular invasion, require treatment with systemic chemotherapy (figure 1) and therefore will be generally excluded from this study unless they refuse the recommended cytoreductive chemotherapy. Patients may have received prior intravesical treatment for their tumor. Patients treated with prior systemic chemotherapy for

urothelial carcinoma are not eligible for this study. Patients with metastatic or surgically unresectable disease are not eligible for this study.

Patient must have no significant co-morbidities interfering with treatment. Patients with interstitial lung disease are not eligible for this trial.

Since tumor response and target inhibition on patient tissue are end-points of the study, patients should agree to have biopsy and cystectomy at M. D. Anderson. Tissue collection is an optional procedure.

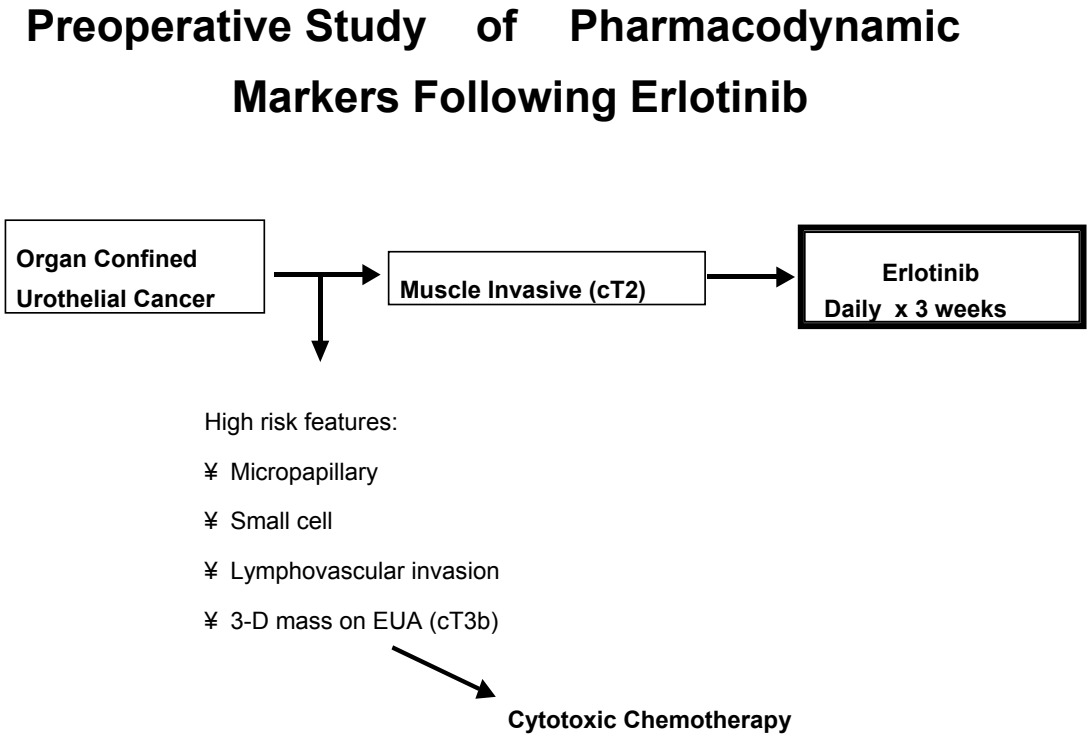
Study Schema:

This is a phase II exploratory study of pre-operative treatment with erlotinib for patients with muscle invasive transitional cell carcinoma. The primary endpoint is pathologic response rate, with additional endpoints regarding target inhibition on patient tissue. Biopsies will be obtained prior to therapy, and residual tumor will be collected at cystectomy.

Treatment Regimen:

Patients will receive erlotinib 150 mg po daily. Patients will receive at least 3 weeks of therapy, scheduled so that the last dose of therapy will be given within 24 hours prior to cystectomy. Since cystectomy will usually be scheduled within 3-5 weeks, therapy will not delay surgery. The treatment may be shortened, if necessary, to accommodate the surgical schedule, but will generally last for 3 weeks.

Figure 1:



3.2 RATIONALE FOR STUDY DESIGN

Current Clinical Practice:

Our current clinical practice for low-risk muscle invasive bladder cancer, is to proceed with cystectomy, and consider additional adjuvant chemotherapy depending upon pathologic stage. Though many of these patients may be cured by surgical resection alone, there is a subset of these patients who relapse and develop incurable metastatic disease. Neoadjuvant cytoreductive chemotherapy is given only for those patients with the following high-risk features: micropapillary tumor, small cell tumor, 3-D mass on EUA, and lymphovascular invasion.

Clinical Endpoint Rationale:

(Please also see section 1.3 for rationale regarding exploring EGFR targeted therapy in bladder cancer).

Though many patients may be cured by surgical resection alone, there is a subset of these patients who relapse and develop incurable metastatic disease. Therefore, while one may not want to put these patients through the toxicity of systemic multi-agent chemotherapy, there is certainly the feeling that something further must be done. This group of untreated patients with intact bladders provides an ideal opportunity to explore new agents and collect tissue specimens to measure candidate prospective markers of response, receptor phosphorylation, and other downstream targets of biological agents.

Use of the pT0 rate has been a well-established surrogate for activity and outcome in the setting of neoadjuvant therapy, and has been employed by our bladder trials group for over a decade of research. Evidence suggests that patients who achieve this surrogate have the highest rates of overall survival, compared to those who do not achieve this clinical endpoint.

Biologic Endpoint Rationale

In addition to the main clinical endpoint, biologic hypotheses will be tested in the studies proposed as part of this trial. The first is that quantification of markers of EMT (E-cadherin, HER-4, and PDGFR-β), and correlation with changes in Ki-67, will allow us to prospectively identify tumors likely to

respond to EGFR-directed therapy. The second is that changes in the expression of active EGFR, cyclin D1, p27, and KI-67 will identify those which are biologically responding to EGFR-directed therapy. The third is that tumor cell death may result from either direct effects on tumor cells or indirect effects of angiogenesis inhibition, so we will also assess the pharmacodynamics of angiogenesis inhibition by measuring the pro-angiogenic factors VEGF and IL-8 as well as MVD, pericyte coverage, and endothelial cell apoptosis by laser scanning cytometry. These studies are important to determine whether angiogenesis inhibition occurs within the same tumors that display growth arrest; it is certainly possible that angiogenesis inhibition occurs more broadly or less frequently than growth arrest, which could have important implications for the early pharmacodynamic assessment of biological benefit from therapy.

Serum and urine levels of MMP-9, IL-8, bFGF, and VEGF will be collected at baseline, weekly (± 3 days) during treatment, prior to cystectomy, following cystectomy, and at the first routine follow-up visit.

In vitro studies suggest that microRNAs are regulators of tumor phenotypes and therapeutic response in bladder cancer. We will also measure free circulating microRNAs in serum or plasma, and in tissue specimens and correlate their expression with protein makers of biologic response, including Ki67, PCNA, and TUNEL. We will also correlate microRNAs to other protein markers related to EGFR sensitivity: ZEB2, ERRF-1, ZEB1, E-cadherin, and TGFalpha. By intercorrelating their results, we hope to derive a comprehensive tumor signature discriminating between EGFR sensitive and resistant tumor.

The overall scientific objective is to define the molecular profile of urothelial carcinoma that responds to EGFR-targeted therapy. Additionally, we will also isolate mRNA, total RNA and microRNA from pre- and post-treatment biopsies for analysis on Affymetrix microarrays, using the institutional core facility (PI: Mini Kapoor, Ph.D.). Ultimately, we hope to use the knowledge gained from this descriptive study to identify appropriate patients for EGFR-directed or other selective targeted therapies.

3.3 SAFETY PLAN

3.3.1 ADVERSE EVENTS USING TARCEVA

Common adverse events associated with Tarceva administration include rash and diarrhea. Other common adverse events include nausea/vomiting, mucositis/stomatitis, headache, and fatigue.

A rash occurred in 75% of Tarceva-treated NSCLC patients enrolled in BR.21. Similar incidence of rash have occurred when erlotinib was administered concurrently with chemotherapy including gemcitabine, paclitaxel/carboplatin, and gemcitabine/cisplatin. A papular, pustular rash manifesting most often on the face and upper trunk was common across all studies, but rash was rarely the cause of study drug discontinuation. Other dermatologic manifestations reported in clinical studies or postmarketing use of Tarceva include nail changes, paronychia, painful fissures or cracking of the skin on the hands and feet, and hair growth abnormalities (alopecia, thinning hair, eyelash/eyebrow changes, hirsutism).

Wearing of contact lenses while receiving Tarceva therapy is not recommended. The incidence of diarrhea in BR.21 was 54% of Tarceva-treated NSCLC patients. The median time to onset of skin rash was 8 days and median time to occurrence of first diarrheal symptom was 9 days.

There have been infrequent reports of serious (including fatal) interstitial lung disease (ILD) in patients receiving Tarceva for treatment of NSCLC or other advanced solid tumors. In Study BR.21, the incidence of ILD (0.8%) was the same in the placebo and Tarceva groups. The overall incidence in Tarceva-treated patients from all studies (including uncontrolled studies and studies with concurrent chemotherapy) is approximately 0.6%. Included in this rate of ILD are reported diagnoses of pneumonitis, radiation pneumonitis, hypersensitivity pneumonitis, interstitial pneumonia, interstitial lung disease, obliterative bronchiolitis, pulmonary fibrosis, acute respiratory distress syndrome, alveolitis, and lung infiltration, irrespective of investigator assessed causality. Most of the cases were associated with confounding or contributing factors such as concomitant/prior chemotherapy, prior radiotherapy, preexisting parenchymal lung disease, metastatic lung disease, or pulmonary infections.

Rare cases of acute renal failure or renal insufficiency have been reported (including fatalities). Many of these cases have been associated with dehydration associated with nausea, vomiting, diarrhea, and/or anorexia. There have been rare reports of renal failure in patients receiving Tarceva in combination with platinum-containing chemotherapy regimens. Febrile neutropenia has been reported in patients receiving concomitant chemotherapy.

Tarceva is both protein bound (92%–95%) and metabolized by hepatic cytochromes CYP3A4 and CYP3A5 and pulmonary cytochrome CYP1A1. Therefore, a potential for drug–drug interaction exists when Tarceva is co-administered with drugs that are highly protein bound or that are CYP3A4 inhibitors/inducers.

Co-administration of Tarceva with omeprazole, a proton pump inhibitor, decreased the exposure of Tarceva (AUC) by 46% and the maximum

concentration (C_{max}) by 61%. There was no change to Tmax or half-life. Therefore, drugs that alter the pH of the GI tract may alter the solubility of Tarceva and hence its bioavailability.

The exposure to Tarceva (AUC) increased to a moderate extent, by 39%, and the maximum concentration (C_{max}) by 17%, when Tarceva was co-administered with ciprofloxacin, an inhibitor of both CYP3A4 and CYP1A2.

Co-administration of Tarceva with an inhibitor of CYP3A4 metabolism (ketoconazole, 200 mg po BID for 5 days) resulted in increased exposure to Tarceva as measured by an 86% increase in median Tarceva AUC and a 69% increase C_{max}, compared with administration of Tarceva alone.

Induction of CYP3A4 metabolism by a known enzyme inducer (rifampin, 600 mg po QD for 7 days) resulted in a 69% decrease in the median Tarceva AUC, compared with administration of Tarceva alone. However, the effect of rifampin on C_{max} was negligible. In another study, rifampicin pretreatment followed by co-administration of rifampicin with a single 450 mg dose of Tarceva resulted in a mean Tarceva exposure (AUC) that was 57.6% of that observed following a single 150 mg Tarceva dose in the absence of rifampicin treatment. Therefore, a potential for drug-drug interaction exists when Tarceva is co-administered with drugs that are highly protein bound or that are potent CYP3A4 inhibitors or inducers.

International normalized ratio (INR) elevations and/or bleeding events have been reported in some cancer patients while on Tarceva alone and in combination with other chemotherapeutic agents, and concomitant NSAIDS or anticoagulants, including warfarin.

3.3.2 General Plan to Manage Safety Concerns

A number of measures will be taken to ensure the safety of patients participating in this trial, addressed through exclusion criteria and routine monitoring. Patients will be evaluated for adverse events at each study visit for the duration of their participation in the study and for 30 days after the discontinuation of Tarceva.

Skin toxicities will be monitored by routine physical examination and managed symptomatically. The following agents may be used to treat rash: diphenhydramine, topical or oral corticosteroids, and topical (clindamycin) or oral antibiotics (tetracycline, minocycline, doxycycline). Topical drying agents are not recommended.

Diarrhea will be monitored and managed symptomatically. Guidelines for management include administration of loperamide and Tarceva dose reduction/interruption as described in Section 4.3.3 and Table 2.

Although quite rare, ILD can be life threatening. Therefore, patients should be monitored closely for symptoms consistent with ILD, such as new onset

dyspnea without an obvious cause. In the event that ILD is suspected, Tarceva treatment should be discontinued and the patient should receive appropriate medical management. Although there is no proven therapy, systemic corticosteroids are often provided. Tarceva should not be restarted in those patients suspected of having drug-related ILD. See Section 4.3.3 and Table 2 for management guidelines, including Tarceva dose interruption.

Liver function abnormalities, including elevated serum ALT, AST, and/or bilirubin, have been observed infrequently with single-agent Tarceva and occasionally with Tarceva in combination with concomitant chemotherapy. Periodic monitoring of liver function is recommended. Tarceva dosing should be interrupted if changes in liver function are severe.

Women of childbearing potential should have a negative pregnancy test prior to starting therapy with Tarceva and should use adequate contraceptive methods during and for at least 2 weeks after Tarceva therapy. Treatment should only be continued in pregnant women if the potential benefit to the mother outweighs the risk to the fetus. If Tarceva is used during pregnancy, the patient should be apprised of the potential hazard to the fetus or potential risk for loss of the pregnancy.

It is not known whether Tarceva is excreted in human milk. Because many drugs are excreted in human milk and because the effects of Tarceva on infants have not been studied, women should be advised against breast-feeding while receiving Tarceva therapy.

3.4 ADMINISTRATIVE STRUCTURE

This is a single institution, investigator sponsored trial performed with the approval of Genentech, Inc. and OSI Pharmaceuticals.

The Department of Genitourinary Medical Oncology will manage this single institution trial along with the infrastructure of the Office of Protocol Research. The trial will follow all MD Anderson Cancer Center Institutional Review Board (IRB) guidelines and policies.

The current requirements of the department include a full time Quality Assurance Program with standard operating procedures (SOP), orientation, as well as staff development. According to the Cancer Therapy Evaluation Program (CTEP), quality assurance includes prevention, detection and action from the beginning of the clinical trial to true completion with analysis and manuscript meeting the overall objectives.

The Quality Assurance program of the Department of Genitourinary (GU) Medical Oncology has been designed to verify compliance with the protocol and regulatory requirements, and identify learning needs of the research staff.

The Departmental program is a supplement the ORERM, and is in alignment with the institutional goals.

3.5 COMPLIANCE WITH LAWS AND REGULATIONS

This study must be conducted in compliance with the protocol and according to Good Clinical Practice as described in the following documents:

The MD Anderson Cancer Center IRB

ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996

US Code of Federal Regulations dealing with clinical studies (21 CFR including parts 50 and 56 concerning informed consent and IRB regulations)

MDA Policy: All protocols involving human subjects will be reviewed and approved by an MDACC Institutional Review Board prior to commencing the research.

45 CFR §46.109 (a) An IRB shall review and have authority to approve, require modification in (to secure approval), or disapprove all research activities covered by this policy.

45 CFR §46.109 (d) An IRB shall notify investigators and the institution in writing of its decision to approve or disapprove the proposed research activity, or of modifications required to secure IRB approval of the research activity. If the IRB decides to disapprove a research activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or in writing.

21 CFR §56.109 (a) An IRB shall review and have authority to approve, require modifications in (to secure approval), or disapprove all research activities covered by these regulations.

21 CFR §56.109 (e) An IRB shall notify investigators and the institution in writing of its decision to approve or disapprove the proposed research activity, or of modification required to secure IRB approval of the research activity. If the IRB decides to disapprove a research activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or writing.

The informed consent process begins when a potential research participant is initially contacted regarding a study by the investigator or his staff. Participants should not be approached about a potential protocol prior to that study being approved by the Institutional Review Board (IRB).

The attending physician shall be responsible for ensuring that the informed consent process is documented by the use of a written consent form approved by the IRB and signed by the participant or participant's legally authorized representative (unless this requirement is specifically waived by the IRB).

Upon activation, each page of the informed consent document is imprinted with the "IRB Approved Consent" stamp. The stamp is signed and dated by the assigned protocol compliance specialists and distributed to the principal investigator. Additional copies must be made and distributed by the department. Consents that are submitted in PDOL will be available on-line following activation.

Only the most recent version of the informed consent document approved by the IRB should be used when addressing new participants. The date stamped on the informed consent document should correspond with the informed consent date in the Protocol Data Management System (PDMS)/Clinical Oncology Research System (CORe) during the registration process, verifying that the correct version of the document has been signed. Consents for protocols that are available on-line through PDOL, will have the most recent revision date typed on the lower right-hand corner of the document.

Informed consent must be obtained prior to the initiation of any protocol-specific screening procedures that are not considered standard of care.

4. MATERIALS AND METHODS

4.1 PATIENTS

4.1.1 INCLUSION CRITERIA

Patients must fulfill all of the following criteria to be eligible for study entry:

1. Patients must have histologic proof of urothelial cancer. This includes bladder cancer, in addition to other tumors of the urothelial lining including renal pelvis, ureteral, and urethral cancer. This group may include any patient requiring cystectomy, including patients with recurrent or extensive superficial disease (cTa-T1N0M0), CIS (carcinoma in situ), or muscle invasive disease (cT2-3aN0M0), whose tumor could not be completely removed at transurethral resection.
2. Patients with the following high-risk features: Micropapillary features (more than focal on pathology); Small cell carcinoma; 3-D mass on exam under anesthesia (EUA); Lymphovascular invasion; Hydronephrosis (unless in the opinion of the treating physician, this is not due to tumor); High grade (grade 3) tumors of the ureter, renal pelvis, or urethra, or

tumors in these areas with radiographic abnormality large enough to recognize as an abnormal mass by CT or MRI imaging; Direct invasion of the prostatic stroma or the vaginal wall (ie: cT4a disease) should be offered neoadjuvant cytoreductive chemotherapy (ie: cisplatin-based). Patients refusing or who are not considered candidates for cytoreductive chemotherapy may be considered eligible. Dr. Siefker-Radtke will be the final arbiter in determining eligibility for the trial.

3.

4. Please note that the presence of variant histologic subtypes is acceptable, except in the case for small cell variant which is traditionally treated with cytoreductive chemotherapy. Patients with small cell who refuse recommended cytoreductive chemotherapy may still be considered eligible.
5. Patients must have an evaluation in the department of urology, and be deemed an acceptable surgical candidate.
6. Patients must NOT have clinical evidence of metastatic disease by either CT or MRI of the abdomen and pelvis, and chest x-ray. In the absence of a bone scan, patients should be free of bone pain and have an alkaline phosphatase < 1.5 x ULN of the upper limit of normal, or a normal bone fraction of alkaline phosphatase. If these features are present, patients should have a bone scan and this should be interpreted as showing no evidence of metastatic disease in order to be eligible.
7. Individuals must be > 18 years of age. In general, urothelial cancer occurs in the 6^{th} to 7^{th} decade of life, so it is unlikely that pediatric patients will be included.
8. Patients, all 18 years and older, must either be not of child bearing potential or have a negative pregnancy test within 2 weeks of treatment. Patients are considered not of child bearing potential if they are surgically sterile (they have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are postmenopausal.
9. Bone marrow function: absolute neutrophil count (ANC) $\geq$ 1,000/ul; platelets $\geq$ 75,000/μl.
10. Renal function: creatinine $\leq$ 2.0 x institutional upper limit of normal (ULN), or a creatinine clearance of ≥ 30 ml/min as calculated by Cockroft-Gault or by 24-hour urine collection.
11. Hepatic function: bilirubin $\leq$ 2.5 x ULN; AST $\leq$ 5.0 x ULN.
12. Zubrod PS $\leq$ 2.
13. Patients with second malignancies are eligible provided that the expected outcome from the second cancer is such that this will not interfere in the

delivery of this therapy, or in doing cystectomy, and provided that the expectation of survival from any prior malignancy is reliably > 4 years.

4.1.2 EXCLUSION CRITERIA

Patients meeting any of the following criteria are ineligible for study entry:

1. Acute hepatitis or known HIV.
2. Active or uncontrolled infection.
3. Significant history of uncontrolled cardiac disease; i.e., uncontrolled hypertension, unstable angina, recent myocardial infarction (within prior 6 months), uncontrolled congestive heart failure, and cardiomyopathy with decreased ejection fraction $\leq$ 40%.
4. Prior therapy specifically and directly targeting the EGFR pathway (i.e.: erlotinib, gefitinib, and cetuximab).
5. Patients with interstitial lung disease.
6. Any concurrent chemotherapy not indicated in the study protocol or any other investigational agent(s).
7. Patients with metastatic or surgically unresectable disease are not eligible for this study. In addition, patients who do not agree to surgery are not eligible for this trial.
8. Patients who have received prior systemic chemotherapy or radiation therapy for urothelial cancer are not eligible. Any prior intravesical chemotherapy is allowed.

4.2 METHOD OF TREATMENT ASSIGNMENT

All patients will receive daily Tarceva. There is no randomization planned, and no placebos will be used. Since this is a single-armed trial, consecutively eligible patients will be offered enrollment.

4.3 STUDY TREATMENT

4.3.1 Formulation

Tarceva oral tablets are conventional, immediate-release tablets containing erlotinib as the hydrochloride salt. In addition to the active ingredient, Tarceva contains lactose (hydrous), microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, and magnesium stearate.

Tablets containing 25 mg, 100 mg, and 150 mg of Tarceva are available. Each bottle will contain 30 tablets, a quantity sufficient for 4 consecutive weeks of dosing, with overage.

For further details, see the Tarceva Investigator's Brochure.

4.3.2 Dosage, Administration, and Storage

Tarceva will be self-administered in an open-label, unblinded manner to all patients enrolled in the study. During the treatment period, patients will receive single-agent Tarceva, 150 mg/day. Tablets should be taken at the same time each day with 200 mL of water at least 1 hour before or 2 hours after a meal. Patients who are unable to swallow tablets may dissolve the tablets in distilled water for administration.

Dose reductions for adverse events will be permitted (see Section 4.3.3). Treatment is continued daily until disease progression or other reason for termination of study therapy (see Section 4.7).

Tarceva tablets will be supplied for clinical trials in white, high-density polyethylene (HDPE) bottles with child-resistant closures and should be stored at temperatures between 15°C and 30°C (59°F and 86°F).

4.3.3 Dose Modification

Dose reduction or interruption of Tarceva for toxicity may take place at any time during the study. Toxicity grading is based on NCI-CTCAE, v 3.0. Dose level reductions are presented in Table 1. If patients do not tolerate the second dose reduction, Tarceva is to be discontinued.

Table 1

Tarceva Dose Level Reductions

Starting Dose	First Reduction	Second Reduction
150 mg/day	100 mg/day	50 mg/day

Dose modification guidelines are summarized in Table 2.

Management of a tolerable Grade 2 or 3 rash should include continuation of Tarceva at the current dose and symptomatic management. If skin rash is intolerable, dose reduction according to Table 2 should be considered. When skin toxicity improves by at least one grade level, the dose may be re-escalated as tolerated. In Phase II trials, this approach enabled dose re-escalation for the majority of patients requiring dose reduction for skin

toxicity. Patients experiencing Grade 4 skin toxicity should be discontinued from study treatment.

For Grade 1 or 2 diarrhea, early intervention should include continuation of Tarceva at the current dose and initiation of loperamide therapy as described in Table 2. Grade 2 diarrhea that persists over 48–72 hours, despite optimal medical management, should be managed by dose reduction according to Table 2. Patients experiencing Grade 3 diarrhea should interrupt Tarceva until resolution to Grade $\leq$1 and re-start at a reduced dose according to Table 2. Patients should be maintained at the reduced dose without attempt at dose re-escalation. Patients experiencing Grade 4 diarrhea should be discontinued from study treatment.

Tarceva should not be restarted in those suspected of having drug-related ILD.

Table 2

Dosage Modification Criteria and Guidelines for Management of Tarceva-Related Toxicities

NCI-CTCAE (v 3.0) Grade	Tarceva Dose Modification	Guideline for Management
Diarrhea		
Grade 1	None	Consider loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until free of diarrhea for 12 hours)
Grade 2	None (Dose reduction of Tarceva is necessary if diarrhea persists over 48–72 hours despite optimal medical management)	Loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until diarrhea free for 12 hours)
Grade 3	Interrupt then dose reduce Tarceva. Tarceva should not be re-escalated.	Interrupt Tarceva until resolution to Grade ≤1, and restart at next reduced dose
Grade 4	Discontinue study treatment.	
Pulmonary Events if possibly ILD		
All Grades	Temporarily interrupt Tarceva pending the diagnostic evaluation. If the pulmonary adverse event is assessed as related to Tarceva, discontinue the patient from study treatment.	Unexplained dyspnea, either new or progressive, should be aggressively evaluated.
Rash		
Grade 1 and 2 Tolerable rash	None	Any of the following: oral antibiotics (tetracycline, minocycline, doxycycline) topical clindamycin, diphenhydramine, topical or oral corticosteroids at discretion of investigator
Grade 3 Intolerable rash	Consider interruption and or dose reduction if unresponsive to symptomatic management. Re-escalation is allowed.	Manage as described above
Grade 4	Discontinue study treatment.	Manage as described above

4.4 CONCOMITANT AND EXCLUDED THERAPIES

Use of anti-neoplastic or anti-tumor agents not part of the study therapy, including chemotherapy, radiation therapy, immunotherapy, and hormonal anticancer therapy, is not permitted while participating in this study.

Use of concurrent investigational agents is not permitted.

There are potential interactions between Tarceva and CYP3A4 inhibitors and CYP3A4 promoters. Although caution and careful monitoring are recommended when use of these compounds is necessary, use of these compounds does not exclude patients from participating in this trial (see Appendix B for a list of CYP3A4 inhibitors and inducers).

Grapefruit juice is a CYP3A4 inhibitor, therefore, consumption of grapefruit or grapefruit juice should be avoided during Tarceva treatment.

The solubility of Tarceva is pH dependent. Tarceva solubility decreases as pH increases. Co-administration of Tarceva with omeprazole, a proton pump inhibitor, decreased the exposure of Tarceva (AUC) by 46% and the maximum concentration (C_{max}) by 61%. There was no change to Tmax or half-life. Therefore, drugs that alter the pH of the GI tract may alter the solubility of Tarceva and hence its bioavailability.

The exposure to Tarceva (AUC) increased to a moderate extent, by 39%, and the maximum concentration (C_{max}) by 17%, when Tarceva was co-administered with ciprofloxacin, an inhibitor of both CYP3A4 and CYP1A2.

Tarceva clearance can be induced by smoking via CYP1A2 induction. Potential drug-drug interaction is expected when Tarceva is taken with CYP1A2 inducers or inhibitors. In a single-dose study in healthy volunteers, the AUC was reduced by 64% in smokers when compared with nonsmokers. In BR.21, current smokers achieved Tarceva trough plasma concentrations that were approximately 2-fold lower than never smokers. Smokers should be advised to stop smoking while taking Tarceva as plasma concentrations of Tarceva are reduced due to the effect of cigarette smoking.

Pretreatment or co-administration of Tarceva did not alter the clearance of a prototypical CYP3A4 substrate, midazolam. Therefore, significant metabolic interactions with other CYP3A4 substrates are unlikely. However, the oral bioavailability of midazolam decreased by up to 24% following Tarceva treatment, which was not attributed to a metabolic interaction.

Patients taking warfarin or other warfarin-derivative anticoagulants should be monitored regularly for changes in prothrombin time or INR.

4.5 STUDY ASSESSMENTS (SEE APPENDIX 1)

4.5.1 SCREENING AND PRETREATMENT ASSESSMENTS

Patients should have a history and physical examination. All patients must be seen by a Urologic Surgeon at M. D. Anderson and have a cystoscopy and exam under anesthesia (EUA) prior to registration. (Please note, an EUA is only done in the setting of urothelial tumors of the bladder).

Laboratory studies: CBC with platelets and automated differential, electrolytes, magnesium, calcium, albumin, LDH, AST (or ALT), alkaline phosphatase, total bilirubin, creatinine, and BUN. Patients should have a prothrombin time if they have not yet had a cysto/EUA. These studies should be obtained within 3 weeks of study entry.

Other baseline studies required at study entry: chest x-ray (within 6 weeks), EKG (within 6 months), CT (or MRI) of the abdomen and pelvis within 6 weeks of study entry. Since the bone scan is known to be falsely negative in a high percentage of cases and is not routinely done in this setting, patients are not required to have a bone scan unless they meet alkaline phosphatase or pain criteria as listed in the inclusion criteria.

Tissue/specimen collection: Cancer tissue will be obtained during cystoscopy (or from archived specimens) and transurethral resection of bladder tumor during routine staging procedures. Tissue following treatment will be obtained at cystectomy

We will also record the time at which the vascular pedicles of the bladder are ligated so we can determine the correlation between ischemic time and integrity of these phosphorylated proteins. Immunohistochemical staining be performed include EGFR, phospho-EGFR, PCNA, CD31, TUNEL, AKT/phospho-AKT, MAPK, and GSK3B. Other markers will be evaluated if appropriate.

Serum and urine levels of MMP-9, IL-8, bFGF, and VEGF will be collected at baseline, weekly ($\pm$ 3 days) during treatment, prior to cystectomy, following cystectomy, and at the first routine follow-up visit.

Pathology specimens and serum and urine samples derived by surgical or other procedures during standard patient care will be stored in the Bladder SPORE tissue bank. Tissue will be appropriately annotated/dated. Requests for tissue will be preferentially used for (1) clinical care, (2) meeting the endpoints of this trial. Additional samples remaining will be made available to investigators interested in studying important molecular targets that may be predictive of outcome or provide additional insight into the mechanism of action of Tarceva or the biology of transitional cell urothelial tumors. Samples

will be collected and stored as per institutional guideline. All requests must be in the context of IRB approved protocols.

4.5.2 EVALUATIONS AND PROCEDURES DURING TREATMENT

All patients will be seen weekly (± 3 days) while on Tarceva by the physician or their designee (research nurse, advance practice nurse, physician assistant, nurse practitioner, clinic nurse) to evaluate for toxicity. Patients will have weekly (± 3 days) CBC with platelets, AST or ALT, serum Cr, and electrolytes.

4.5.3 TREATMENT DISCONTINUATION ASSESSMENTS

Patients discontinuing treatment for toxicity, will be seen every other week by the physician or their designee (research nurse, advance practice nurse, physician assistant, or nurse practitioner) to document improvement, until the time of surgery. Patients discontinuing treatment by choice alone (ie: in the setting of no toxicity), will not be required to follow weekly.

4.5.4 FOLLOW-UP ASSESSMENTS

Patients should be seen in the clinic or contacted by telephone to determine if any serious or non-serious adverse events have occurred within 30 days (±3 days) of termination of Tarceva dosing.

Patients will have routine post-surgical follow-up after cystectomy as determined by their urologist. Long-term follow-up, including CT of the abdomen and pelvis, CXR, and laboratory tests will be done as determined by the treating physician. Long-term follow up will be done every 6 months for the first year, followed by yearly intervals for the next 4 years. Follow-up may be done more frequently based upon the pathologic stage/discretion of the treating physician. Patients may go on to adjuvant chemotherapy if deemed appropriate by their treating physician. They will continue to be followed for 4 years.

4.6 ASSAY METHODS

Patients will be treated with daily Tarceva for 3 weeks, followed by cystectomy (or ureterectomy/nephrectomy/urethrectomy). The last dose of Tarceva will be given within 24 hours prior to scheduled surgical time.

Patients will have tissue collected at baseline, prior to Tarceva, as part of staging cystoscopy (or from archived specimens), and will have residual tumor tissue collected at the time of cystectomy (or ureterectomy/nephrectomy). Blood and urine specimens will also be

collected at baseline, weekly (± 3 days) while on treatment, at cystectomy, and at the first restaging follow-up visit (usually within 3-6 months).

Markers of the epithelial to mesenchymal transition (EMT) (ie: E-cadherin, ZEB1, ZEB2, ERRF-1, TGFα, HER4, PDGFR-beta, vimentin, and fibronectin) will be analyzied in pre- and post-treatment biopsies to correlate expression patterns with clinical response (ie pT0 rate) and biological response and target inhibition. EMT expression patterns will be correlated with markers of anti-proliferation (KI-67), apoptosis (TUNEL) and angiogenesis (two-color TUNEL, phosphor-receptor, and microvessel density). EGFR copy number and mRNA expression in pre and post-treatment biopsies using Affymatrix arrays and tumor tissue and serum microRNAs and Total RNA will also be correlated with changes with changes observed with EMT, growth arrest, and apoptosis. To provide further insight into biology, we will also interrogate membrane (phosphorylated EGFR) and downstream receptor signaling pathways (ERKs, AKT.mTOR, GSK-3beta).

4.7 PATIENT DISCONTINUATION

Patients may discontinue study treatment at any time. Any patient who discontinues treatment will be encouraged to return to the study center to undergo treatment discontinuation assessments. The primary reason for discontinuation should be recorded. Reasons for discontinuation of a patient by the investigator include, but are not limited to, the following:

- Documented disease progression
- Clinically significant deterioration of the patient's condition prior to treatment discontinuation
- Patient noncompliance
- Persistent (≥3 weeks) NCI-CTCAE version 3.0 Grade 3 or Grade 4 adverse event or any significant adverse event that compromises the patient's ability to participate in the study
- Investigator determination that it is not in the patient's best interest to continue participation
- Pregnancy
- Patient Decision

4.8 STATISTICAL METHODS

Determination of Sample and Monitoring for Efficacy

Sample Size: Clinical Endpoint

Assume the baseline response (pT0 rate) to be 10%, and an increase of the response rate to 25% after the neoadjuvant therapy. With type I and type II error rates of 0.1 and 0.1, we need a maximum number of 40 patients for the Simon's Min-max two-stage design. At the first stage of the trial, if there are 2 or less responses observed among the first 27 patients, the trial should be terminated and the treatment will not be considered for further study. If there are at least 3 responses observed among the first 27 patients, 13 more patients will be enrolled in the second stage for a total of 40 patients. If at least 7 responses are observed from all 40 patients, the treatment will be considered for further investigation. To take into consideration of possible unevaluable patients in assessing the biologic endpoints, we plan on accruing a total of 42 patients.

Safety Endpoints and Monitoring:

Toxicity outcomes will be monitored periodically for patients after the treatments. Termination will be recommended if it is very likely that the surgery-limiting toxicity rate has exceeded 15%. For this trial, surgery-limiting toxicity will be defined as hemorrhage requiring major non-elective intervention or wound dehiscence (defined as fascial disruption with evisceration) within 30 days post-operatively. [Formally, stop if Prob ($\theta > 0.15$) ≥ 0.80, where θ refers to the toxicity rate for the investigated regimen. The prior distribution on θ is Beta(.1, .9)]. The trial will be terminated if the following stopping boundaries are crossed:

Number of patients	Stop trial if number of toxicity greater than or equal to
5	2
10	3
15	4
20	5
25	6
30	7
35	8

40	9

Note that the trial may be terminated prior to reaching the specified number of patients in a cohort. For example, if there are two patients with toxicity among the first 3 patients, the trial will be terminated for excessive toxicity.

A simulation study was conducted to examine the property of the stopping rule under a variety of scenarios (10,000 simulated trials per scenario). The following table summarizes the probability of stopping the trial early due to toxicity and the expected sample size for the indicated true toxicity rate**:**

True toxicity rate	Prob(stop early)	percentage of sample size		
		25%	50%	75%
0.05	0.03	40	40	40
0.10	0.15	35	40	40
0.15	0.37	10	40	40
0.20	0.62	5	20	40
0.25	0.82	5	10	20
0.30	0.93	5	10	15

Moreover, the operating characteristics of the joint monitoring rules for both efficacy and toxicity are provided in the following table under the assumption that the two distributions are independent to each other. The true response rates are listed in the first row, and the true toxicity rates in the first column. The marginal probability that the trial is terminated at the first stage for futility is shown in the second row, while the toxicity is not considered.

Tox/Resp	0.1	0.2	0.25	0.3	0.35
0	0.48	0.07	0.02	0.005	0.001
0.05	0.496	0.098	0.049	0.035	0.031
0.1	0.558	0.21	0.167	0.154	0.151
0.15	0.672	0.414	0.383	0.373	0.371
0.2	0.802	0.647	0.628	0.622	0.62
0.25	0.906	0.833	0.824	0.821	0.82
0.3	0.964	0.935	0.931	0.93	0.93

4.8.1 Analysis of the Conduct of the Study

Methods for detection of violations of enrollment criteria, major protocol violations and deviations can be found in section 6.5. Reasons for patient discontinuation from the study will be documented, as per section 4.7

4.8.2 Analysis of Demographic and Baseline Characteristics

Demographic characteristics will be collected at baseline. These should included age, gender, Zubrod performance status, date of initial diagnosis, any prior treatment including resection and prior intravesical therapy. Descriptive analysis will be performed.

4.8.3 Efficacy Analysis

The primary endpoint of this trial is to estimate the response rate (ie: pT0 rate) of patients with urothelial cancer treated with erlotinib prior to cystectomy. In this context, response will be defined as the absence of residual cancer in the resected specimen. Since this is a single institution study, all specimens will be reviewed by a genitourinary pathologist in the department of pathology at M. D. Anderson Cancer Center.

Use of the pT0 rate has been a well-established surrogate for activity and outcome in the setting of neoadjuvant therapy, and has been employed by our bladder trials group for over a decade of research. Evidence suggests that patients who achieve this surrogate have the highest rates of overall survival, compared to those who do not achieve this clinical endpoint.

A secondary objective is to estimate the 4-year disease-free survival of patients with urothelial cancer treated with erlotinib prior to cystectomy.

An interim analysis will be performed after the first 27 patients. If there are 2 or less responses among the first 27 patients, the trial will be terminated. If there are 3 or more responses, the trial will continue accrual.

Full details regarding the analysis plans can be found in section 4.9.7

4.8.4 Safety Analysis

Patients will be monitored for toxicity using NCI CTCAEv3.0. Toxicity will be monitored at the weekly (± 3 days) visits while on therapy. Dose reductions based upon toxicity can be found in section 4.3.

Surgery will not be delayed for missed Tarceva doses. Patients who miss a dose of Tarceva may resume treatment if timing permits. However, if the toxicity will interfere with or delay surgery, Tarceva will be discontinued.

Though treatment with this agent is generally expected to be well-tolerated, patients will be monitored for specific toxicities which would be of concern in the surgical setting. For this trial, surgery-limiting toxicity will be defined as hemorrhage or wound dehiscence within 30 days post-operatively. The statistics employing the early stopping rules can be found in section 4.8.

4.8.5 Pharmacokinetic Analyses

This will be a descriptive study and extent of target inhibition will be correlated with clinical outcomes, including tumor response. In this setting, a 50% decrease in KI-67 will serve as a measure of antiproliferation, with a sample size of 42, the response rate can be estimated within 15% of the true value with 95% confidence interval. In the initial phase of analysis, we will apply a univariate analysis for each of the markers: E-cadherin, HER4, PDGFR-β, GSK3β, phosphorylation, cyclin D1, p27 and KI67 expression. We will estimate the change in proportion of each marker expression after treatment with a 95% confidence interval. We will also evaluate correlations among these markers before and after the treatment.

These events will be correlated with any effects of therapy on MVD, mean vessel volume, changes in the percentages of tumor pericytes, endothelial cells, or both, and tumor and stromal cell apoptosis. Extent of target inhibition (EGFR signaling) will be correlated with KI67 as an indicator of response, because the study design does not allow us to measure objective tumor response. Angiogenesis factor expression will be correlated with endothelial cell apoptosis and MVD.

4.8.6 Missing Data

Patients receiving at least 1 dose of study medication will be assessable for both response and toxicity. Patients who come off study without having received any of the study therapy will be replaced.

4.9 DATA QUALITY ASSURANCE

Clinical information regarding registration and off study will be entered to the Institutional required data base CORe and PDMS. Clinical data for analysis will be entered to the departmental database GURU. The Data Base Coordinator will work with the protocol specific team in order to generate reports for quality analysis.

5. ASSESSMENT OF SAFETY

The safety of Tarceva will be assessed through collection and analyses of adverse events (AEs) and laboratory tests. These assessments will occur weekly (± 3 days) while on therapy. In addition, patients will be assessed for potential surgical toxicity occurring within 30 days of cystectomy. Though this treatment is expected to be generally well-tolerated, specific therapy-limiting toxicity can be found in section 4.8.

5.1 SPECIFICATION OF SAFETY VARIABLES

Safety assessments will consist of monitoring and recording protocol-defined adverse events (AEs) and serious adverse events (SAEs); measurement of protocol-specified hematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug(s).

Death as a result of disease progression is only to be assessed as efficacy measures and not as AEs or SAEs.

5.1.1 Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational (medicinal) product or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with urothelial cancer that were not present prior to the AE reporting period (see Section 5.2.1)
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as biopsies)
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period
- Diagnoses and/or symptoms associated with urothelial cancer should be reported as AEs if they worsen or change in character. Clinical progression of urothelial cancer should not be reported as an AE.

5.1.2 Serious Adverse Events

An AE should be classified as an SAE if:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above).

All AEs that do not meet any of the criteria for serious should be regarded as nonserious AEs.

The terms "severe" and "serious" are not synonymous. Severity (or intensity) refers to the grade of a specific AE, e.g., mild (Grade 1), moderate (Grade 2), or severe (Grade 3) myocardial infarction (see Section 5.2.2). "Serious" is a regulatory definition (see previous definition) and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations from the Sponsor to applicable regulatory authorities.

Severity and seriousness should be independently assessed when recording AEs and SAEs on the CRF.

5.2 METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, as outlined in Section 5.1.1, are recorded on the CRF and reported to the Sponsor in accordance with protocol instructions.

Death as a result of disease progression endpoints are only to be assessed as efficacy measures and not as AEs or SAEs.

5.2.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

SAEs that are observed or reported prior to initiation of study treatment should be recorded as SAEs on the CRF if they are associated with protocol-mandated interventions (e.g., invasive procedures such as biopsies, medication washout, or no treatment run-in).

5.2.2 Assessment of Adverse Events

Investigators will assess the occurrence of AEs and SAEs at all patient evaluation timepoints during the study. All AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be recorded in the patient's medical record and on the appropriate AE or SAE CRF page.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity (see Table 3), regulatory seriousness criteria if applicable, suspected relationship to the investigational product (see following guidance), and actions taken.

The AE grading (severity) scale found in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 3.0, will be used for AE reporting.

Table 3

Adverse Event Grading (Severity) Scale

Grade	*Severity*	*Alternate Description* [a]
1	*Mild (apply event-specific NCI-CTCAE grading criteria)*	*Transient or mild discomfort (<48 hours); no interference with the patient's daily activities; no medical intervention/therapy required*
2	*Moderate (apply event-specific NCI-CTCAE grading criteria)*	*Mild to moderate interference with the patient's daily activities; no or minimal medical intervention/therapy required*
3	*Severe (apply event-specific NCI-CTCAE grading criteria)*	*Considerable interference with the patient's daily activities; medical intervention/therapy required; hospitalization possible*
4	*Very severe, life threatening, or disabling (apply event-specific NCI-CTCAE grading criteria)*	*Extreme limitation in activity; significant medical intervention/therapy required, hospitalization probable*
5	*Death related to AE*	

Note: Regardless of severity, some events may also meet regulatory serious criteria. Refer to definitions of an SAE (see Section 5.1.2).

[a] *Use these alternative definitions for Grade 1, 2, 3, and 4 events when the observed or reported AE is not in the NCI-CTCAE listing.*

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

- **YES**

There is a plausible temporal relationship between the onset of the AE and administration of the investigational product, and the AE cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the investigational product; and/or the AE abates or resolves upon discontinuation of the investigational product or dose reduction and, if applicable, reappears upon re-challenge.

- **NO**

Evidence exists that the AE has an etiology other than the investigational product (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal

relationship to administration of the investigational product (e.g., cancer diagnosed 2 days after first dose of study drug).

Note: The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the "Yes" or "No" causality assessment for individual AE reports, the Sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities.

5.2.3 SAE Reporting Guidelines

All STEAEs will be recorded on an MDACC Internal Adverse Event Form and faxed to:

Genentech Drug Safety

Fax: (650) 225-4682 or (650) 225-4683

AND:

Arlene Siefker-Radtke, M.D.

MD Anderson Cancer Center

1155 Pressler Street, Unit 1374

Houston, TX 77030

Follow-up information:

Additional information may be added to a previously submitted report by completing a new MDACC Internal Adverse Event Form and indicating "F/U

Follow-Up" in the appropriate field.

Occasionally, Genentech may contact the reporter for additional information, clarification or current status of the subject for whom an adverse event was reported. For questions regarding STEAE reporting, you may contact the Genentech Drug Safety representative noted above.

5.3 PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

5.3.1 Eliciting Adverse Events

A consistent methodology of non-directive questioning for eliciting AEs at all patient evaluation timepoints should be adopted. Examples of non-directive questions include:

"How have you felt since your last clinical visit?"

"Have you had any new or changed health problems since you were last here?"

5.4 EXPEDITED REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS

Investigators will submit written reports of all SAEs, regardless of attribution, to Genentech within 48 hours of learning of the events. For initial SAE reports, investigators should record all case details that can be gathered within 48 hours. The completed SAE form and SAE Fax Cover Sheet should be faxed immediately upon completion to Genentech's Drug Safety Department at:

(650) 225-4682
or
(650) 225-5288

Relevant follow-up information should be submitted to Genentech's Drug Safety as soon as it becomes available and/or upon request.

5.5 TYPE AND DURATION OF FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

All AEs and SAEs that are encountered during the protocol-specified AE reporting period should be followed to their resolutions, or until the investigator assesses them as stable, or the patient is lost to follow-up. Resolution of AEs and SAEs (with dates) should be documented on the appropriate AE page and in the patient's medical record to facilitate source data verification.

For some SAEs, the Sponsor or its designee may follow-up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report). Contact information is as follows:

PI: Arlene O. Siefker-Radtke, MD

Assistant Professor of Genitourinary Medical Oncology

1155 Hermann Pressler, Unit 1374

Houston, TX 77030

Phone: 713-792-2830

Fax: 713-745-1625

6 SPONSOR AND INVESTIGATOR REQUIREMENTS

Sponsors are responsible for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation(s), ensuring that the investigation(s) is conducted in accordance with the general investigational plan and protocols contained in the IND, maintaining an effective IND with respect to the

investigations, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug.

6.1 STUDY INITIATION

Before the start of this study and the shipment of investigational agent to the main site and any sub-site, the following documents must be on file at Genentech, Inc.

- Current curricula vitae and license of the Principal Investigator
- Final Protocol and ICF
- A signed and dated investigator brochure acceptance form
- Written documentation of IRB approval of protocol and ICF (identified by title and date of approval) for each site
- A signed Confidentiality Agreement
- A signed Clinical Trial Agreement for each site
- A letter of cross-reference to the OSI Pharmaceuticals IND

6.2 STUDY COMPLETION

The following data and materials are required by Genentech before a study can be considered complete or terminated:

- Copies of protocol amendments and IRB approval/notification, if appropriate
- Copies of the IRB final report, documentation of submission to the IRB and to the FDA.
- A summary of the study prepared by the Principal Investigator (Study report, manuscript and/or abstract)
- All regulatory documents (e.g., updated curriculum vitae for each Principal Investigator, updated U.S. FDA Form 1572 for each site)

6.3 INFORMED CONSENT

The informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative. If applicable, they will be provided in a certified translation of the local language.

Original signed consent forms must be filed in the site study binder or in each patient's study file.

6.4 INSTITUTIONAL REVIEW BOARD APPROVAL

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. In addition, any advertising materials must be approved by the IRB. The study will be conducted in accordance with U.S. FDA, applicable national and local health authority, and IRB requirements.

The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant adverse events.

Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the Investigator Brochure and that are considered possibly or probably related to the study drug by the investigator. Some IRBs may have other specific adverse event requirements to which investigators are expected to adhere. Investigators must immediately forward to their IRB any written safety report or update provided by Genentech (e.g., IND safety report, Investigator Brochure, safety amendments and updates, etc.).

6.5 STUDY MONITORING REQUIREMENTS

An important part of routine clinical trial management is scheduled reporting. All trials must have and annual review every 365 days per CFR312.33. The Office of Protocol Research will send a reminder notice 60 days prior to the anniversary of the last review. The assigned research team must be able to report all protocol related deaths, routine AEs, SAEs.

Monitoring, accrual, eligibility evaluability, deviations, violations

Protocol specific checklists will be developed for the eligibility and protocol compliance review. The following will be audited from both source and database entry:

a. Informed consent
b. Eligibility/Evaluability
c. Stratum or randomization
d. Trial Management or Treatment (correct dose/schedule/therapy)
e. Scheduled assessments such as labwork and diagnostic tests
f. Toxicities/adverse events (AEs)/serious adverse events (SAEs)
g. Response if appropriate, stopping rules or safety assessments as written
h. Off study evaluation
i. Data verification against source

Department Audit and Monitoring

Each protocol not already monitored by a sponsor or the ORERM will be a priority for Departmental monitoring or audit. The cases for audit will be chosen by reviewing the overall accrual. A minimum of 10% patient accrual will be assessed. The long term goal of the program is to audit 25% of patient accrual.

Once the patients are chosen we will audit a minimum of 1 course of therapy. The goal is to audit 30% of the protocol assessment points but the minimum will be 10%, or registration date in comparison to treatment date, consent form, eligibility criteria, and a full cycle of therapy.

6.6 DATA COLLECTION

Data collection for this trial will meet the required elements noted within the trial.

6.7 STUDY MEDICATION ACCOUNTABILITY

All study drug required for completion of this study will be provided by OSI-Pharmaceutical (manufacturer), in partnership with Genentech (*unless otherwise noted*). The recipient will acknowledge receipt of the drug by

returning the drug receipt form indicating shipment content and condition. Damaged supplies will be replaced.

Study drug accountability records should be maintained by the site in accordance with the regulations.

The original drug supply request of Tarceva™ will be submitted to Genentech along with the form "Approval for Drug Re-Supply", indicating which personnel will be able to submit drug re-supply requests.

All subsequent drug re-supply requests will be directly submitted to OSI-Pharmaceuticals from the site.

At the time of study closure, the unused, used and expired study drug will be destroyed at the site per Institutional SOPs and OSI should be provided with documentation when this has occurred.

6.8 DISCLOSURE OF DATA

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. This medical information must be made available to Genentech and authorized representatives of Genentech, upon request, for source verification of study documentation.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, local health authorities, Genentech, Inc, OSI Pharmaceuticals and their authorized representative(s), collaborators and licensees, and the IRB for each study site, if appropriate.

6.9 RETENTION OF RECORDS

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

7. REFERENCES

1. Cerny T, Barnes DM, Hasleton P, Barber PV, Healy K, Gullick W, et al. Expression of epidermal growth factor receptor (EGF-R) in human lung tumours. Br J Cancer 1986;54:265–9.
2. Davies DE, Chamberlin SG. Targeting the epidermal growth factor receptor for therapy of carcinomas. Biochem Pharmacol 1996;51:1101–10.
3. DeVita VT, Hellman S, Rosenberg SA, editors. Cancer: principles and practice of oncology. 6th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2001.
4. Fontanini G, Vignati S, Bigini D, Mussi A, Lucchi H, Angeletti CA, et al. Epidermal growth factor receptor (EGFr) expression in non–small cell lung carcinomas correlates with metastatic involvement of hilar and mediastinal lymph nodes in the squamous subtype. Eur J Cancer 1995;31A:178–83.
5. Fossella F. Docetaxel in second-line treatment of non-small-cell lung cancer. Clin Lung Cancer 2002:3 Suppl 2:S23–8.
6. Fujino S, Enokibori T, Tezuka N, Asada Y, Inoue S, Kato H, et al. A comparison of epidermal growth factor receptor levels and other prognostic parameters in non–small cell lung cancer. Eur J Cancer 1996;32A:2070–4.
7. Gatzemeier U., Pluzanska A., Szczesna A., Kaukel E., Roubec J., Brennscheidt U., De Rosa F., Mueller B., Von Pawel J.. TALENT: Results of a phase III trial of erlotinib (OSI-774) combined with cisplatin and gemcitabine (GC) chemotherapy in advanced non-small cell lung cancer (NSCLC). ASCO 2004; Abstract 7010
8. Herbst R. S., Prager D., Hermann R., Miller V., Fehrenbacher L., Hoffman P., Johnson B., Sandler A. B., Mass R., Johnson D. H. TRIBUTE: A phase III trial of erlotinib HCl (OSI-774) combined with carboplatin and paclitaxel (CP) chemotherapy in advanced non-small cell lung cancer (NSCLC). ASCO 2004; Abstract 7011.
9. IMPATH Inc. Analysis of EGFr Expression in a selection of tumour types. IMPATH Study Number PFZ04. 1998-1999:1–16.
10. Jamal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics. CA Cancer J Clin 2003;53:5–26.

11. Lei W, Mayotte JE, Levitt ML. Enhancement of chemosensitivity and programmed cell death by tyrosine kinase inhibitors correlates with EGFR expression in non-small cell lung cancer cells. Anticancer Res 1999;19:221–8.

12. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:1838–49.

13. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlations with clinical response to gefitinib therapy. Published 29 April 2004. http://www.sciencemag.org/cgi/rapidpdf/1099314v1.pdf (accessed 17 May 2004).

14. Pfeiffer P, Clausen PP, Andersen K, Rose C. Lack of prognostic significance of epidermal growth factor receptor and the oncoprotein p185HER-2 in patients with systemically untreated non-small-cell lung cancer: an immunohistochemical study on cryosections. Br J Cancer 1996;74:86–91.

15. Miller VA, Kris MG, Shah N, Patel J, Azzoli C, Gomez J, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non–small-cell lung cancer. J Clin Oncol 2004:22:1103–9.

16. Reissmann PT, Koga H, Figlin RA, Holmes EC, Slamon DJ. Amplification and overexpression of the cyclin D1 and epidermal growth factor receptor genes in non–small-cell lung cancer. Lung Cancer Study Group. J Cancer Res Clin Oncol 1999;125:61–70.

17. Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E. Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non–small cell lung cancer but does not predict tumor progression. Clin Cancer Res 1997;3:
515–22.

18. Rusch V, Mendelsohn J, Dmitrovsky E. The epidermal growth factor receptor and its ligands as therapeutic targets in human tumors. Cytokine Growth Factor Rev 1996;7:133–41.

19. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183–232.

20. Sekine I, Takami S, Guang SG, Yokose T, Kodama T, Nishiwaki Y, et al. Role of epidermal growth factor receptor overexpression, K-ras point mutation and c-myc amplification in the carcinogenesis of non-small cell lung cancer. Oncol Rep 1998;5:351–4.

21. Shepherd FA, Dancey J, Ramlau R, Mattson K, Gralla R, O'Rourke M, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000;18:2095–103.

22. Shepherd FA, Pereira J, Ciuleanu TE, Tan EH, Hirsh V, Thongprasert S, Bezjak A, Tu D, Santabarbara P, Seymour L. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st line or 2nd line chemotherapy. A National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) trial. Proceedings of ASCO 2004; Abst 7022.

23. Veale D, Kerr N, Gibson GJ, Kelly PJ, Harris AL. The relationship of quantitative epidermal growth factor receptor expression in non-small cell lung cancer to long term survival. Br J Cancer 1993;68:162–5.

24. Veale D, Ashcroft T, Marsh C, Gibson GJ, Harris AL. Epidermal growth factor receptors in non–small cell lung cancer. Br J Cancer 1987;55:513–6.

APPENDIX A

Study Flowchart

Assessment	Screening/ baseline	Weekly (± 3 days)	At cystectomy	Follow-up
Informed Consent	X			
Medical history	X[a]			X
Physical examination	X			X
Vital signs	X	X		X
Performance status	X			X
BSA	X			
Tumor staging	X			X
CBC with differential and platelets	X	X		X
Serum Chemistries	X[b]	X[c]		
Chest x-ray, CT or MRI of abdomen and pelvis, and EUA	X[d]			X
Bone Scan	X[e]			
EKG (within 6 months)	X			
Pregnancy test	X			
Toxicity		X		X
Tumor specimen for research	X		X	
Blood and urine specimens for research	X	X	X	X
Cystectomy			X	

a Tobacco/smoking history, and exposure to chemicals
b Electrolytes, BUN, serum creatinine, total bilirubin, alkaline phosphatase, AST or ALT, Ca, albumin, LDH
c Only serum creatinine, AST or ALT and electrolytes.
d CXR, CT or MRI, and EUA will be done within 6 weeks of study entry. (EUA only done in the setting of urothelial tumors of the bladder, and will not be repeated routinely during follow-up).
e Only if alkaline phosphatase > 1.5 X ULN

APPENDIX B

List of CYP3A4 Inhibitors

From http://www.georgetown.edu/departments/pharmacology/davetab.html

The following are known inhibitors of CYP3A4:

Delaviridine	Indinavir
Nelfinavir	Ritonavir
Saquinavir	Amiodarone
Cimetidine	Ciprofloxacin
Clarithromycin	Diethyl-dithiocarbamate
Diltiazem	Erythromycin
Fluconazole	Fluvoxamine
Gestodene ++	Grapefruit juice
Itraconazole	Ketoconazole
Mifepristone	Nefazodone
Norfloxacin	Norfluoxetine
Mibefradil	Troleandomycin
Atazanavir	Indinavir
Telithromycin	Voriconazole

The following are known inducers of CYP3A4:

Rifampicin	Phenytoin
Rifabutin	Rifapentine
Carbamazepine	Phenobarbital
St. John's Wort	

APPENDIX C

ECOG Performance Status Scale

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework or office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours
3	Capable of only limited self-care, confined to a bed or chair >50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead