TITLE: A Phase I Study of AZD6244 in Combination with Capecitabine and Radiotherapy in Locally Advanced Adenocarcinoma of the Rectum

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Capecitabine, Clinical Center Pharmacy, NIH

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Précis

Background:

- Local recurrences of rectal cancer are morbid and difficult to manage effectively.
- Colorectal cancers frequently harbor *RAS mutations* and EGF/EGFR over-expression.
- AZD6244 is an orally available selective, adenosine triphosphate–uncompetitive inhibitor of MEK1/2 that sensitizes tumor cells to radiation *in vitro* and *in vivo*.

Objectives:

Primary

- To define the maximum tolerable dose of AZD6244 Hyd-Sulfate delivered BID, 7 days per week, in combination with radiation therapy (RT) and Capecitabine in patients with locally advanced adenocarcinoma of the rectum without distant metastases.
- To define the dose-limiting toxicities and toxicity profile associated with administration of AZD6244 Hyd-Sulfate delivered BID, 7 days per week in combination with RT and Capecitabine

Secondary

- To evaluate the pharmacokinetics of AZD6244 delivered alone and in combination with Capecitabine 825 mg/m^2 PO BID.
- To obtain exploratory information regarding the pathologic response rate obtained after treatment with the MTD of AZD6244 Hyd-Sulfate in combination with Capecitabine and 50.4 Gy of RT.
- To determine if changes in phosphorylated ERK (pERK) in peripheral blood mononuclear cells correlates to changes in pERK in rectal tumors in the setting of treatment with AZD6244.
- To perform an exploratory analysis to determine if the presence of activating mutations in *RAS* or *BRAF* in tumor or changes in plasma transforming growth factor-α (TGFα) levels and tumor pERK/total ERK with AZD6244 treatment alone and after AZD6244 in combination with Capecitabine and RT predicts for down staging or pathologic response.

Eligibility:

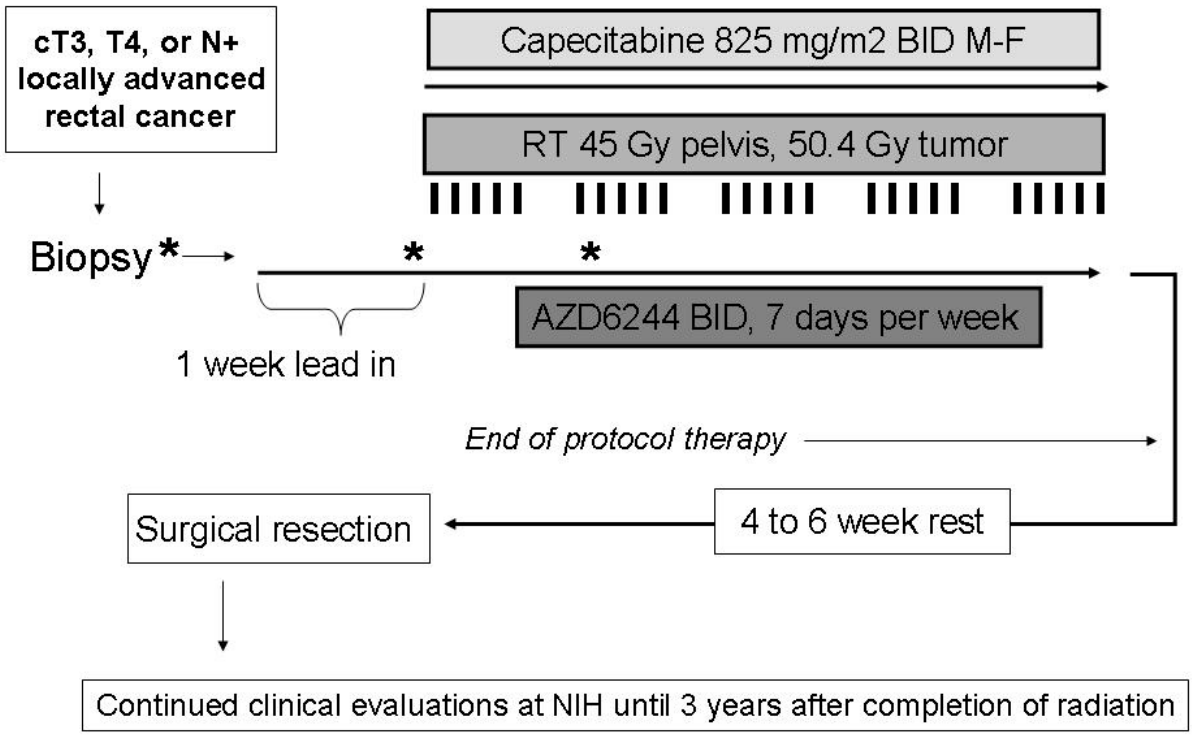
- Histologically or cytologically confirmed locally advanced, non-metastatic adenocarcinoma of the rectum (clinical stage T3AnyN, T4AnyN, or AnyTN+).
- Age $\geq$18 years.
- ECOG performance status $\leq$2.
- Normal organ and marrow function.

Design:

- All patients will receive 50.4 Gy of RT to the pelvis and rectal tumor delivered concurrently with Capecitabine and AZD6244. AZD6244 will be delivered BID daily, 7 days per week, in a dose escalated fashion. AZD6244 will begin one week prior to Capecitabine and RT and will conclude on the last day of Capecitabine and RT.
- Capecitabine will be delivered at 825 mg/m2 PO every 12 hours, 5 days per week, starting on the first day of RT and continuing until the last day of RT.
- Biopsies of tumor tissue will be obtained prior to treatment, after one week of AZD6244, and

after one week of AZD6244, RT, and Capecitabine for correlative assays.

SCHEMA


cT3, T4, or N+ locally advanced rectal cancer
Capecitabine 825 mg/m2 BID M-F
RT 45 Gy pelvis, 50.4 Gy tumor
Biopsy*
*
*
AZD6244 BID, 7 days per week
1 week lead in
End of protocol therapy
Surgical resection
4 to 6 week rest
Continued clinical evaluations at NIH until 3 years after completion of radiation

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1. OBJECTIVES

1.1. Primary Objectives

- To define the maximum tolerable dose of AZD6244 Hyd-Sulfate delivered BID, 7 days per week, in combination with radiation therapy and Capecitabine in patients with locally advanced adenocarcinoma of the rectum without distant metastases.

- To define the dose-limiting toxicities and toxicity profile associated with administration of AZD6244 Hyd-Sulfate delivered BID, 7 days per week in combination with radiation therapy and Capecitabine

1.2. Secondary Objectives

- To determine if changes in phosphorylated ERK (pERK) in peripheral blood mononuclear cells correlates to changes in pERK in rectal tumors in the setting of treatment with AZD6244.

- To obtain exploratory information regarding the pathologic complete response rate (pCR) obtained after treatment with the MTD of AZD6244 Hyd-Sulfate in combination with Capecitabine and 50.4 Gy of radiation.

- To determine if a reduction in phosphorylated ERK (pERK) in peripheral blood mononuclear cells correlates to similar changes in ERK phosphorylation in rectal tumors in the setting of treatment with AZD6244.

- To perform an exploratory analysis to determine if the presence of activating mutations in *RAS* or *BRAF* in tumor or changes in plasma transforming growth factor-α (TGFα) levels and tumor pERK/total ERK with AZD6244 treatment alone and after AZD6244 in combination with Capecitabine and radiation predicts for tumor/nodal down staging or pCR

2. BACKGROUND

2.1 AZD6244

AZD6244 (ARRY-142886) is a potent, selective, orally-available, and non-ATP competitive small molecule inhibitor of the mitogen-activated protein (MAP) kinase kinase, MEK-1/2.(1) AZD6244 inhibited the activity of purified MEK enzyme with an IC_{50} of 10-14 nM, and was found to be inactive or minimally active at 10 μM against a panel of other kinases, including epidermal growth factor receptor (EGFR), erbB2, p38α, ERK2, and MKK 6 kinases. Because ERK is the only known substrate of MEK, the inhibition of MEK will target only the ERK signal transduction pathway and other signal transduction pathways will not be blocked. AZD6244 is metabolized to biologically active N-desmethyl AZD6244 which is more potent than the parent compound. *In vitro*, *in vivo* and preliminary results from clinical studies suggest that AZD6244 exhibits a favorable pharmacologic and toxicologic profile.

The RAS/RAF/MEK/ERK signaling pathway plays a central role in the regulation of many cellular processes including proliferation, survival, differentiation, apoptosis, motility, and metabolism.(2, 3) This pathway is one of the most important and best understood MAP kinase signal transduction pathways, activated by a diverse group of extracellular signals including integrins, growth factor receptors (*i.e.,* EGFR, platelet-derived growth factor receptor [PDGFR], and insulin-like growth factor-1 receptor), and cytokines.(4) Activated RAS triggers the phosphorylation and activation of RAF kinase which then phosphorylates MEK1 and MEK2 on 2 serine residues.(5) Activated MEK phosphorylates its only known substrates, ERK1 and ERK2. Phosphorylated ERK dimerizes and translocates to the nucleus(6) where it is involved in several important cellular functions, including cell proliferation.

Overexpression of growth factors or growth factor receptors involved in the RAS/RAF/MEK/ERK pathway and activating genetic mutations of the signaling proteins may lead to uncontrolled proliferation and tumor formation. For example, RAS genes are the most frequently mutated oncogenes detected in human tumors.(4) RAS proteins are guanine nucleotide binding proteins that activate RAF proteins when bound to GTP. Cancer-associated mutations in RAS proteins stabilize the GTP-bound form of RAS, thereby providing a constitutive signal downstream in the cascade. In addition to being found in almost all pancreatic adenocarcinomas, RAS mutations are found in ~50% of colorectal carcinomas, 25-50% of lung adenocarcinomas, and also in some breast or ovarian cancers. *BRAF* mutations have also been observed in many human cancers, particularly melanoma (30-60%), thyroid (30-50%), colorectal (5-20%), and ovarian (~30%) cancers.(7) These mutations in *BRAF* usually involve gain-of-function substitutions that render the kinases constitutively active. Also, studies of primary tumor samples and cell lines have shown constitutive activation or over-activation of the MAP kinase pathways in cancers of the pancreas, colon, lung, ovary, and kidney.(8) Therefore, agents targeting the RAS/RAF/MEK/ERK pathway may inhibit oncogenic signaling in tumor cells.

2.1.1 Nonclinical Studies

2.1.1.1 Efficacy

In vitro studies have shown that AZD6244 and its N-desmethyl metabolite are potent and selective inhibitors of MEK.(9) However, significant biochemical activity was not detected when the two compounds were tested against a diverse panel of 305 other molecules, including enzymes, receptors, kinases, transporters, and ion channels. The effects of AZD6244 on ERK phosphorylation and cell viability were determined in a panel of cell lines in which the mutational status of RAF and RAS are known. AZD6244 inhibited ERK1 and ERK2 phosphorylation with IC_{50}s ranging from 0.0018 to 0.041 μM. AZD6244 was particularly potent in inhibiting the cell viability of cell lines with V600E *BRAF* gene mutation and some cell lines with *KRAS* mutations. The N-desmethyl metabolite was found to be 3-fold more active than the parent compound in inhibiting ERK phosphorylation, and 5-fold more potent in inhibiting cell viability.

Significant suppression of tumor growth in response to AZD6244 treatment was

observed in several xenograft mouse models derived from a range of tumor types including melanoma, breast, pancreatic, lung, colon, and hepatocellular carcinomas.(1, 9) In the Calu-6 lung cancer xenograft model, AZD6244 suppressed tumor growth at doses of 10, 25, or 100 mg/kg given twice daily, and the minimal effective dose was identified as 0.75 mg/kg administered twice daily. In this model, MEK activity was inhibited as assessed by determination of phosphorylated ERK (pERK) levels in tumor. Studies using human colorectal xenograft models (SW620, Colo205) demonstrate that AZD6244 inhibits tumor growth by inhibition of cell proliferation and by induction of apoptosis.

2.1.1.2 Pharmacokinetic/Pharmacodynamic Studies

Oral bioavailability of AZD6244 was favorable in rats (37% at 10 mg/kg) and monkeys (86% at 1 mg/kg), but bioavailability decreased with increasing dose.(9) The declining bioavailability at higher doses may be due to the low aqueous solubility of AZD6244. No accumulation of AZD6244 was observed following multiple dosing in rats or monkeys. Studies in rats indicate that AZD6244 is widely distributed, although tissue concentrations were lower than blood concentrations. High levels of protein binding (93.7-99.7%) were observed in all preclinical species tested and in humans (98.4%). AZD6244 was excreted rapidly within 48 hours via the feces.

The value of pERK as a biomarker of AZD6244 efficacy has been explored in nonclinical studies. In the Calu-6 human lung cancer xenograft model, levels of pERK in the tumors were determined by both immunohistochemical (IHC) staining of formalin-fixed tissue sections and by western blot analysis of tumor protein lysates.(9) Following an acute tumor-suppressive dose of 25 mg/kg of AZD6244, immunostaining for pERK in the cytoplasm was reduced by approximately 90% at 1, 2 and 4 hours post-dose, and recovered to >50% of the level of the control by 24 hours. The increase in tumor pERK content by 24 hours correlated with the decrease in plasma concentration of AZD6244. The same trend was observed by western blot analysis. These data showed that tumor pERK levels are a potential biomarker for AZD6244 activity *in vivo*.

A whole blood fluorescence-activated cell sorting (FACS) assay was developed to monitor pERK levels in peripheral blood mononuclear cells (PBMCs) to allow assessment of AZD6244 activity without tissue biopsy.(9) Whole blood samples from cynomolgus monkeys at pre-dose and 1, 2, 4, 8, 12 hours post-dose were analyzed for pERK levels. Plasma concentrations of AZD6244 correlated with inhibition of TPA-induced ERK phosphorylation. Similarly, the *ex vivo* addition of AZD6244 to human whole blood from volunteers resulted in the inhibition of ERK phosphorylation in isolated PBMCs. Data from an ongoing phase 1 study in cancer patients also indicate that there is a strong correlation between plasma drug concentration and inhibition of ERK phosphorylation in blood from these patients, demonstrating the effectiveness of pERK as a mechanistic biomarker for MEK inhibition in the clinical setting.(10)

2.1.1.3 Toxicologic Studies

The toxicologic effects of AZD6244 were evaluated in acute dose (single or two doses on a single day) and 1-month, repeat-dose studies in Sprague-Dawley rats and cynomolgus monkeys.(9) The repeat-dose study in rats indicated that the agent was well tolerated but produced soft stools and gastrointestinal mucosal mineralization associated with increased serum phosphorus and decreased albumin. Diarrhea, dehydration, electrolyte imbalance, and secondary renal changes were observed in monkeys. The vehicle, Captisol® (SBE-CD), used in the formulation is known to cause gastrointestinal disturbances in preclinical studies. A reduction in the volume of Captisol® administration to monkeys was associated with a reduced incidence of diarrhea; however, it is likely that AZD6244 exacerbated the vehicle effects. The no observable adverse effect levels (NOAEL) in rats and monkeys in the 1-month studies were identified as 10 mg/kg/day and 20 mg/kg/day (administered twice daily), respectively. AZD6244 and its active metabolite showed no evidence of mutagenic potential, but AZD6244 produced an increase in micronucleated immature erythrocytes in mice, predominantly via an aneugenic mode of action.

Safety pharmacology studies were performed to assess the effects of AZD6244 and its active metabolite on key organ systems.(9) The agents did not produce any toxicologically significant effect. Evidence of gastric mucosal lesions and minor increases in airway resistance were observed in some rats following a 100 mg/kg dose; however, these effects were not apparent in the 1-month study.

2.1.2 Clinical Experience

The clinical experience is summarized from the AZD6244 Investigator Brochure Version 8.(11) A number of clinical studies with AZD6244 have been completed or are ongoing. As of November 26, 2008 studies ARRY-0401, D1532C00003, D1532C00005, D1532C00008, D1532C00011, and D1532C00012 have been completed and D1532C00004 and D1532C00020 are ongoing. In addition, six monotherapy trials of AZD6244 free-base are sponsored by the NCI for which no efficacy data is yet available.

AZD6244 was initially dosed as a free-base suspension which was first evaluated in a single two-part, open-label, multicenter phase 1 company-sponsored study (ARRY-0401).(9) An MTD of 100 mg BID was achieved and subsequent Phase II trials with this preparation utilized this dosing regimen. In the Phase II study D1532C00003 samples for plasma concentration analysis were collected. In the Phase II study D1532C00005 the pharmacokinetics and relative bioavailability of the Hyd-Sulfate preparation compared to the free base suspension were evaluated. The MTD for the Hyd-Sulfate preparation was determined to be 75 mg BID. Further development of AZD6244 is intended to continue with the Hyd-Sulfate preparation.

2.1.2.1 Pharmacokinetics

N-desmethyl is the active metabolite of AZD6244, which is 3 to 5 times more potent to the parent compound. An amide metabolite can also be detected in human plasma but is 40 to 50 fold less active than AZD6244. Single dose pharmacokinetics of AZD6244 and N-desmethyl have been characterized following dosing of both the free base suspension (study ARRY-0401) for dose levels between 50 to 300 mg and the

Hyd-Sulfate preparation (D1532C00005) for dose levels between 25 to 100 mg. The parameters described below are those obtained with the Hyd-Sulfate preparation. AZD6244 was absorbed relatively quickly across dose levels, with a median t_{max} of 1.5 hours. Plasma N-desmethyl AZD6244 concentrations/exposure followed a similar pattern to AZD6244 over time, although C_{max} and AUC values were <15% of the parent within each patient. Following the peak, AZD6244 concentrations decreased multi-exponentially with a mean half-life (t½) of 5 to 7 hours. The t½ of the N-desmethyl metabolite was 9 to 13 hours. Limited data is available for AZD6244 amide. Concentrations of this metabolite were variable within subjects and between subjects. The time to maximum concentration was highly variable among patients.

Study D1532C00020 evaluated the pharmacokinetics of AZD6244 in patients under fasting conditions and after a high fat meal. Glsmean Cmax and AUC were reduced by approximately 60% and 20% under fed conditions compared to exposure following AZD6244 taken fasted. There was a trend toward a later t_{max} in the fed condition. Based on these findings it is recommended that AZD6244 should be taken on an empty stomach.

Plasma pharmacokinetic parameters were similar for single and a multiple dosing (day 8, 15, and 22), suggesting minimal accumulation over time with twice daily dosing, consistent with the terminal half-life observed. A similar pharmacokinetic profile was observed on day 8 compared to day 1 supporting that the steady state was achieved in this time. The exposure of AZD6244 N-desmethyl followed a similar pattern. Exposure on multiple dosing was comparable within patients. Comparison of data from 7 or 14 days BID dosing suggest steady state was achieved by day 7.

2.1.2.2 Safety

The safety data presented reflects monotherapy safety data from ongoing and completed trials with both formulations of AZD6244. It should be noted that there may be a different safety and tolerability profile for AZD6244 co-administered with other anti-cancer agents.

Dermatological adverse events are frequently reported in association with administration of AZD6244, with dermatitis acneiform being the most commonly reported event starting within the first month of treatment. Other types of dermatological events occur only after a more prolonged period of administration with AZD6244, including skin fissures and palmar-plantar eythrodysaethesia (hand-foot syndrome). Where the dermatological AE is CTCAE Grade 3 or above, or is intolerable CTCAE Grade 2, a dose holiday (either with or without dose reduction) may be required to bring the CTCAE intensity to within tolerable limits, although this may not result in total resolution of the symptoms whilst the patient is continuing to receive AZD6244. An algorithm for additional interventions for the management of dermatological adverse events has been adapted from clinical experience of management of similar dermatological effects of the EGFR tyrosine kinase inhibitor agents.(12, 13) The co-administration of AZD6244 with other anti-cancer agents, some of which are associated with dermatological adverse events, may result in a

higher frequency or intensity of these adverse events. Dermatologic adverse events have led to withdrawal of one patient from each of four AZD6244 studies (4 patients total).

Diarrhea, nausea, and vomiting are consistent adverse event in AZD6244 trials. The majority of events were CTCAE grade 1 for diarrhea and nausea or CTCAE grade 1 or 2 for vomiting. Fewer than 2% of patients reported a SAE of diarrhea, nausea, or vomiting with the exception that in the D1532C00005 study with the Hyd-Sulfate preparation SAE vomiting was reported by 7% of patients. Most diarrhea events started within 2 weeks of BID dosing. Approximately one third of patients with diarrhea required concomitant loperamide to manage diarrhea. Approximately half of patients with nausea adverse events required concomitant medication to control symptoms. The co-administration of AZD6244 with other anti-cancer agents, some of which are associated with gastrointestinal adverse events, may result in a higher frequency or intensity of these events.

Adverse events of fatigue are reported across all studies with AZD6244. In the majority of cases the causality was split approximately equally between study drug and the disease under study. A higher frequency of fatigue AEs were reported with the Hyd-Sulfate preparation compared to the free-base suspension in Phase II monotherapy trials (67.9% vs 20.8%). A minority of fatigue events were CTCAE grade 3 with the highest frequency of 14% of patients in study D1532C0005 including 1 DLT.

Edema (primarily peripheral and periorbital) and fluid accumulation has been reported consistently in studies of AZD6244 with peripheral edema being most common. The underlying etiology of the edema and fluid accumulation adverse events is unclear at present. There is currently no evidence to suggest that the edema is due to congestive cardiac failure. Most peripheral edema AEs were CTCAE grade 1. The time of onset was commonly several weeks into treatment with AZD6244. Some patients required initiation of diuretic therapy to control the peripheral edema.

Some patients receiving AZD6244 have been observed to develop asymptomatic decreases in LVEF in the absence of obvious confounding comorbidities. The co-administration of AZD6244 with other anti-cancer agents, some of which are known to be associated with cardiotoxicity, may be associated with an increased risk of impairment of cardiac function. There have been four AE reports of left ventricular dysfunction, two reports of ejection fraction decreased, and one report each of ventricular dysfunction and ventricular hypokinesia in patients receiving AZD6244 free-base. Two events of left ventricular dysfunction were considered SAEs. In study D153200005 with the Hyd-Sulfate preparation LVEF was monitored and data indicate a trend toward a drop in LVEF across all dose cohorts by week 8 assessments. No comparator data is available and the study population included patients who may have received prior cardiotoxic chemotherapy.

Adverse events of dyspnea have been reported across trials of AZD6244.

Seven SAEs of dyspnea have been reported from trials of AZD6244 and all subjects had thoracic disease and/or disease progression at the time of the report. In study D1532C00005 with the Hyd-Sulfate preparation dyspnea and exertional dyspnea were reported by 3.8% and 28.6% of patients which are higher frequencies than observed with the free-base formulation. Treatment related dyspnea events were generally reported several weeks after the start of BID dosing and the majority were CTCAE grade 1 to 2. Hypoxia AEs all originated from a single study site at altitude. Three events of hypoxia were reported as treatment-related SAEs. There has been one treatment-related SAE of pneumonitis but no other AEs of pneumonitis have been reported in any other completed study. Preliminary unvalidated data indicates that one patient from study D1532C0020 has reported SAEs of lung infiltration and interstitial lung disease.

Increases in SBP and DBP have been observed in clinical studies with AZD6244. AEs of hypertension have been reported by some patients treated with AZD6244. In study D1532C00003 increases in blood pressure were observed after one week of treatment, with mean increases at 8 weeks of treatment of 7.4 mmHg SBP and 5.3 mmHg DBP. Hypertension was reported as an AE in 8 patients receiving AZD6244. An additional 8 patients from studies D1532C0008, D1532C0011, and D1532C0012 reported AEs of hypertension. In D1532C00005 small increases in SBP and DBP were observed consistently in the first week of treatment and hypertension was reported as an AE in 14.3% of patients. Hypertension AEs in these studies were generally CTCAE grade 1 or 2 but resulted in initiation of antihypertensive medications in some patients.

Adverse events relating to visual function have been reported at a low frequency in all studies. There were no specific treatment emergent structural findings reported from those patients that underwent an ophthamological evaluation after reporting a visual disturbance adverse event. There were anecdotal reports of asymptomatic raised intra-ocular pressure at the protocol mandated Week 4 eye test in one study. The events were reported under a variety of terms with the most common being blurred vision but also including visual disturbance (red spots, uneven floor, intermittent shadows, dark spots in viewing area), photopsia, diplopia, and altered visual depth perception. The majority of visual AEs were CTCAE grade 1 in intensity. One AE of visual disturbance in D1532C0005 was CTCAE grade 3 in intensity, starting on day 2 of treatment but resolved fully without intervention four days later.

One of the patients on D1532C00020 with raised intraocular pressure went on to report CTCAE grade 2 blurred vision followed by an SAE of convulsion which occurred two days after insertion of a biliary stent. CT and MRI scans gave a differential of Reversible Posterior Leukencephalopathy Syndrome (RPLS) in combination with preexisting vascular abnormalties. No other cases of suspected RPLS have been reported with AZD6244.

Increases in the serum liver transaminases, ALT and AST have been observed in clinical trials with AZD6244. Mean increases were observed within 1 week of

AZD6244 treatment and did not continue to rise beyond levels reached by the end of the first 4 weeks of treatment, except in patients at time points immediately prior to withdrawal due to disease progression. The majority of reported transaminase elevations either remained within normal limits or increased by no greater than 1 CTCAE grade. In Study D1532C00005 an increase in ALP was observed. However, no significant trends have been observed for bilirubin in any study, with either formulation. Regular measurements of AST, ALT and other liver function parameters will continue to be monitored and the health care professionals involved in clinical studies should look for signs of liver toxicity in patients receiving AZD6244. All patients with an aspartate aminotransferase (AST, SGOT), alanine aminotransferase (ALT, SGPT) or bilirubin value above upper limit of normal at the time of the last dose of AZD6244 should have a further liver chemistry profile (AST, ALT, bilirubin and alkaline phosphatase) performed 30 days after permanent discontinuation of AZD6244 in order to fully document reversibility.

In the melanoma Phase II study (D1532C00003) an increase in serum phosphate was observed in some patients after initiation of AZD6244 when compared with patients randomized to temozolomide. One patient on AZD6244 required therapeutic intervention initiated due to a calcium:phosphate product which exceeded 4.5 mmol/L. Regular monitoring of serum calcium and phosphorus levels, and the calcium:phosphate product is advised in clinical studies with AZD6244.

In Study D1532C00005, data shows a slight decrease in platelet count, however this was not considered clinically significant. No other clinically significant changes in hematological parameters were identified across the clinical study program. However, hematological events are potential risks, particularly when AZD6244 is administered in combination with other anti-cancer therapies.

2.1.2.3 Clinical Activity

The following discussion summarizes the single agent efficacy data. There is no efficacy data for the combination of AZD6244 and capecitabine

ARRY-0101 was a Phase I, open-label, multicenter study was designed primarily to investigate the safety and tolerability of the free-base suspension formulation of AZD6244. Objective tumor response evaluation using RECIST criteria indicate that the best overall response in this study was stable disease. Of the patients who had at least one pre- and postdose measurement of target lesions recorded, stable disease was observed in 17 patients. Long term (≥5 months) stable disease was observed in 9/57 patients, 6 of whom had melanoma.

Study D1532C00003, a Phase II, open-label, randomized study assessed the safety and efficacy of AZD6244 vs temozolomide (TMZ) in patients with unresectable AJCC stage 3 or 4 malignant melanoma in 200 randomized patients (104 for AZD6244 and 96 for TMZ).(14) In the overall population there was no statistically significant difference between the 2 treatment groups for the primary endpoint of PFS

and OS may have been improved in the TMZ treatment arm compared to those treated with AZD6244. These data may be confounded by the high frequency of patients crossing-over from TMZ to AZD6244 in this study. Six patients receiving AZD6244 had a confirmed partial response (PR), of which 5 were *BRAF*+. Eleven patients randomized to TMZ had a confirmed response (10 PRs and 1 complete response [CR]), 3 of which (all PRs) were *BRAF*+.

Study D1532C00008, a Phase II, open-label, randomized study assessed the efficacy and safety of AZD6244 vs capecitabine in 70 patients (38 for AZD6244 and 32 for capecitabine) with advanced or metastatic pancreatic cancer who had failed first-line gemcitabine therapy. There was no statistically significant difference between the 2 treatment groups for the primary endpoint of time to death (TTD). The median TTD was 164 days and 152 days in the AZD6244 and capecitabine groups, respectively. Results were similar for the secondary outcome variable of progression event count. Five patients (2 patients in the AZD6244 group and 3 patients in the capecitabine group) achieved a best overall response of PR, as defined by the investigator (unconfirmed). Because tumor response was not an endpoint of the study and a single assessment of PR was accepted as a best overall response of PR, these are not considered robust data for response.

Study D1532C0001 was a Phase II, open label, randomized study that assessed the efficacy and safety of AZD6244 versus capecitabine in 69 patients.(15) There was no significant difference between treatments in the primary endpoint of the number of patients with a disease progression event (82% patients in the AZD6244 group versus 80% patients in the capecitabine group). There was no evidence of a statistically significant difference in PFS between the groups. Overall best response by RECIST criteria was stable disease (10 patients) for AZD6244 while there was 1 patient with PR and 15 with stable disease in the capecitabine group).

Study D1532C00012 was a Phase II, open-label, randomized study that assessed the efficacy and safety of AZD6244 versus pemetrexed in 84 patients (40 for AZD6244 and 44 for pemetrexed) with NSCLC who had failed one or two prior chemotherapeutic regimens.(16) The primary objective of this study was to assess the efficacy of AZD6244 versus pemetrexed in the second or third line treatment of advanced NSCLC. There was no statistically significant difference between treatments in the primary endpoint of the number of patients with a disease progression event: 28 (70%) patients in the AZD6244 group versus 26 (59%) patients in the pemetrexed group. There was no difference in PFS between treatment groups. At the time of the final analysis, four patients had achieved a best overall response of CR (1 patient in the pemetrexed group) or PR (2 patients in the AZD6244 group, 1 patient in the pemetrexed group) to treatment.

Study D1532C0005 was a Phase I, open label, multi-center study to assess the safety, tolerability and pharmacokinetics of a capsule formulation of AZD6244 in patients with advanced solid malignancies who have failed standard therapy or for which no standard therapy exists.(17) Efficacy assessment in this study was an exploratory

endpoint. Part A of the study (31 patients) was a dose escalation study designed to provide adequate tolerability, safety, pharmacokinetic, and pharmacodynamic data. In Part A, the doses investigated were 25 mg, 50 mg, 75 mg and 100 mg delivered twice daily. Part B (29 patients randomized) was designed to determine the relative oral bioavailability of the Hyd-Sulfate capsule and to expand the safety, tolerability and preliminary efficacy profile of the MTD from Part A (75 mg BID). In Part B, patients received either a single dose of capsule formulation or free-base suspension formulation on Day 1 and 8, followed by continuous twice daily dosing of capsule formulation from Day 9 onwards. Fifty-five patients had RECIST evaluable tumors. There was one complete response in the 75 mg dose cohort in Part A after 16 weeks. This patient had a *BRAF*+ melanoma. At the75 mg dose 16/35 (45.7%) patients had stable disease for ≥6 weeks. Nine patients in Part A and 13 patients in Part B had a best response of stable disease. Ten out of 55 (18.2%) patients (not including the patient who had a CR) had stable disease of ≥16 weeks. Seven patients in Part A and 12 patients in Part B had a best response of progressive disease, and 10 patients in Part A and 3 patients in Part B were not evaluable for response.

In summary, the results of four Phase II clinical studies with the free base suspension formulation as monotherapy compared to standard chemotherapy agents did not demonstrate superior efficacy of either agent. Objective responses were seen in both the melanoma and NSCLC studies. In the melanoma study D1532C00003, 5/6 of the partial responses were in patients with the *BRAF* mutation, and in addition a durable complete response has been seen in study D1532C00005 with the Hyd-Sulfate capsule formulation in a patient with *BRAF*+ melanoma. In the NSCLC phase II monotherapy study no tumor samples were available for either patient who experienced a partial response.

2.1.2.4 Pharmacodynamics

The effect of AZD6244 on the phosphorylation of ERK in peripheral blood mononuclear cells was evaluated in Part A of study ARRY-0401. Using FACS analysis, the magnitude of ERK phosphorylation relative to baseline was determined in TPA-stimulated pre- and post-treatment blood samples. AZD6244 inhibited TPA-induced ERK phosphorylation at an IC_{50} of approximately 134 ng/mL (total concentration in plasma). The magnitude of inhibition was found to correlate with plasma AZD6244 concentrations. Further analyses using the active metabolite will be carried out in the future.

Preliminary, unvalidated data from paired tumor biopsies pre- and post-dose (4 hours post-dose on ~ Day 15) were obtained from patients in Part B. Formalin-fixed, paraffin embedded tissue samples were evaluated by immunohistochemistry for phosphorylated extracellular signal-regulated kinase - pERK (score range 0-400), proliferative index - Ki-67 (range 0-100%) and apoptosis by cleaved caspase 3 (0-100%). Evaluable pre and post biopsies were obtained from a range of tumors for Ki67 (20 patients), pERK (19 patients) and cleaved caspase 3 (16 patients). Two patients were dosed at 200mg, the remaining at 100mg. At the tolerated dose of 100 mg, tumor pERK staining was reduced compared to untreated specimens, with a g-

mean reduction in nuclear staining of 81% (90% CI 50% to 93%) compared to pre-dose. A reduction in Ki-67 was seen in 9/20 patients with a reduction greater than or equal to 50% of pre-dose seen in 5/20 patients. No increases greater than 1% were seen on the apoptotic marker cleaved caspase 3.

2.1.2.5 Potential Drug Interactions

In vitro metabolic studies of AZD6244 using hepatocytes from humans and animals found that the biologically active N-desmethyl derivative was detected in mouse and human hepatocytes, was detected minimally in monkeys; and was not detected in rats (Investigator's Brochure, 2006). Cytochrome P450 (CYP) 1A2 was the enzyme primarily responsible for the formation of the N-desmethyl derivative; CYP2C19 and CYP3A4 were also minimally involved in the transformation. Neither AZD6244 nor its active metabolite were found to be inhibitors of CYP isoforms 1A2, 2C8, 2C19, 2D6, or 3A4. However, AZD6244 was found to be a weak inhibitor of CYP2C9 (IC_{50} = 44.7 uM) and the N-desmethyl derivative was a weak inhibitor of CYP1A2 (IC_{50} = 18.9 uM). Thus, at the systemic AZD6244 concentrations observed following 100mg AZD6244 mix and drink formulation in man, no significant cytochrome P450 interactions would be expected. Since the formation of N-desmethyl AZD6244 from AZD6244 may occur through the CYP 1A2 pathway and smoking induces this pathway, the smoking status of the subjects should be recorded in all studies (*i.e.*, smoker or non-smoker) to investigate whether smoking status influences systemic drug exposures of N-desmethyl AZD6244.

2.2 **Other Agent(s)**

Capecitabine and radiation are frequently combined for the treatment of gastrointestinal malignancies. Capecitabine is a fluoropyrimidine carbamate that is an orally administered prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR) that is converted to 5-fluorouracil (5-FU). 5'-DFUR is hydrolyzed to the active drug 5-FU by thymidine phosphorylase which is present in many tissues in the body. Thymidine phosphorylase is expressed in higher concentrations in some human carcinomas compared to surrounding tissues.

In the setting of rectal cancer, there is a growing experience of Capecitabine and radiotherapy in the neoadjuvant setting.(18) The maximum tolerated dose of Capecitabine is 800-825 mg/m2 twice daily combined with 50.4 Gy of radiation.(19-22) Downstaging occurs in approximately 50-85% of patients receiving the combination of Capecitabine and radiation in the neoadjuvant setting for locally advanced rectal cancer. Pathologic complete response rates of 8-16% have been described with the combination, similar to that observed with the combination of 5-FU and radiation in this setting.(23, 24) The most common Grade 3 or higher toxicity observed with Capecitabine in this setting is diarrhea which may be observed in as many as 20% of patients.

2.3 **Locally Advanced Adenocarcinoma of the Rectum**

Over 40,000 cases of adenocarcinoma of the rectum occurred in the United States in 2008.(25) Patients with locoregionally advanced disease (T3, T4, and N positive) are at a high risk of local recurrence if treated with surgery alone. Combined modality therapy with

chemotherapy and radiation improve survival and local control after surgical resection of locally advanced rectal cancer.(26-28) Treatment of rectal cancer with chemotherapy and radiation in a neoadjuvant setting further improves local control and reduces toxicity compared to adjuvant chemoradiation.(23) Currently, the standard of care in the United States for patients with locally advanced (T3-T4N0, N+) rectal cancer is combined chemoradiation followed by surgical resection with additional adjuvant chemotherapy based on stage and risk factors. Because local recurrences are morbid and difficult to manage effectively, radiation is being evaluated with new agents delivered alone or with additional adjuvant therapy to improve outcomes and reduce toxicity.

2.4 **Rationale**

Colorectal cancers frequently harbor a common set of well-described genetic abnormalities. For example, in clinical colorectal cancer specimens, the prevalence of *RAS mutations* is 40%,(29) the prevalence of EGF/EGFR over-expression is 44%,(30) and the prevalence of BRAF mutations is approximately 14%.(31) The high prevalence of mutations resulting in activation of the mitogen-activated protein kinase (MAPK) cascade via activating Ras mutations in addition to the prevalence of pathway activation through autocrine and paracrine mechanisms (i.e. EGF/EGFR) presents a unique therapeutic target. The availability of novel small molecule inhibitors to elements of the MAPK signal transduction pathway provides an opportunity to target Ras oncogene addition and EGFR pathway addiction as a therapeutic intervention.

In addition, the Ras-MAPK pathway is of particular interest in the context of radiotherapy for a number of reasons. Activation of the Raf-MEK-ERK pathway occurs rapidly in tumor cells after exposure to ionizing radiation. (32-34) Activation of the Ras-Raf-MEK-ERK cascade though mutations in Ras and Raf is known to result in enhanced tumor cell proliferation and enhanced survival after irradiation. (35-37) Furthermore, inhibition of Ras and Raf in cell lines with activating Ras mutations results in sensitization to ionizing radiation. (37-39) These data suggest that inhibition of the Ras-Raf-MEK-ERK cascade may sensitize cells to ionizing radiation.

Preclinical work in the Radiation Oncology Branch at the NCI has shown that AZD6244 enhances radiation induced cell death and regrowth delay in a variety of cell lines both *in vitro* and *in vivo*. (40) For *in vivo studies*, AZD6244 was delivered to groups of 5 to 8 tumor bearing nude mice by oral gavage four hours prior to irradiation with a single fraction of 3 Gy (Figure 1). Mice were followed individually and tumor volumes were measured at least twice weekly. To obtain a dose enhancement factor comparing the tumor radiation response in mice with and without AZD6244 treatment, the normalized tumor growth delays were calculated, which accounts for the contribution of AZD6244 to tumor growth delay induced by the combination treatment. Normalized tumor growth delay was defined as the time in days for tumors to grow from 172 to 1,500 mm^3 in mice exposed to the combined modality minus the time in days for tumors to grow from 172 to 1,500 mm^3 in mice treated with AZD6244 only. The dose enhancement factor, obtained by dividing the normalized tumor growth delay in mice treated with AZD6244 + IR by the absolute growth delay in mice with A549 tumors treated with radiation only, was 3.38 for 50 mg/kg of AZD6244. The dose enhancement factor for the addition of AZD6244 in the MiaPaCa2

xenograft model was 2.3.

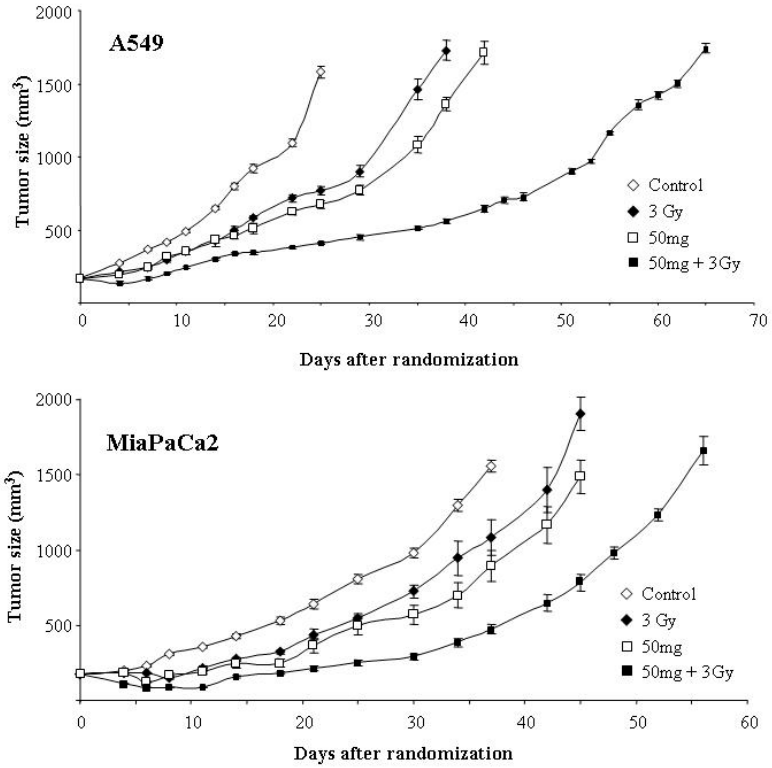


Figure 1. The effects of AZD6244 on A549 and MiaPaCa2 xenograft tumors. When tumors reached 177 mm3 in size, mice were randomized into four groups: vehicle, AZD6244, radiation, or AZD6244 plus radiation. AZD6244 was dissolved in water and given by oral gavage at 50 mg/kg delivered in a single dose. Radiation (3 Gy) was delivered 4 hours after the AZD6244 treatment. Each treated group contained 5 mice with the vehicle control group containing 10 mice. From Chung et al.(40)

Further preclinical work has shown that AZD6244 abrogates the G2 cell cycle checkpoint and enhances mitotic catastrophe following ionizing radiation.(40) In addition, no enhancement of radiation response was seen in fibroblast cell lines (Figure 2, unpublished data) suggesting that AZD6244 may selectively sensitize tumor cells to radiation.

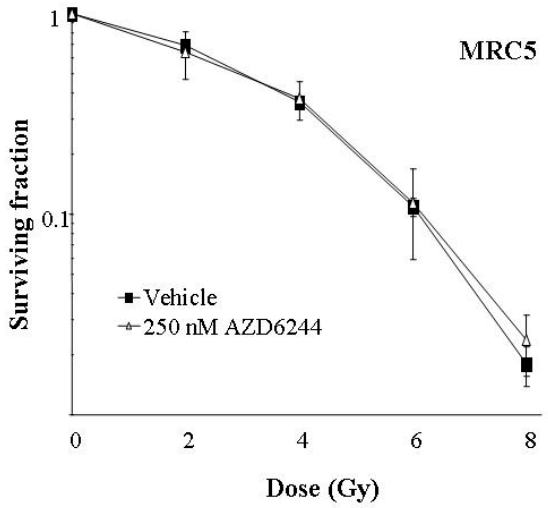
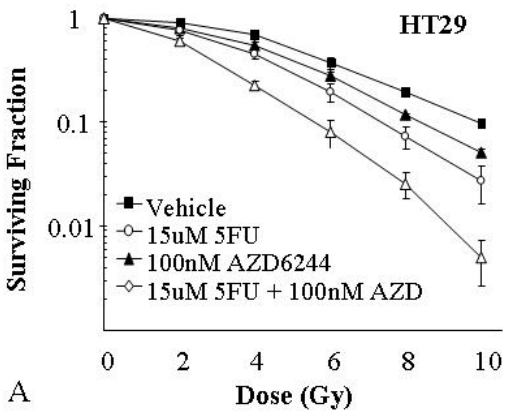
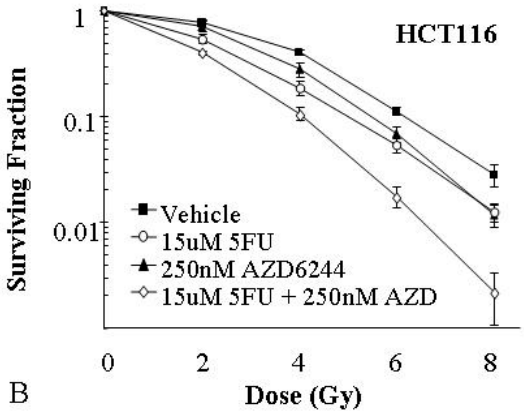
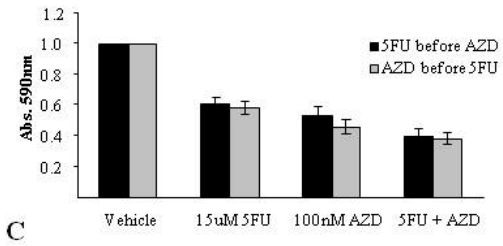


Figure 2. The effects of AZD6244 on the radiation response of MRC5 fibroblasts. MRC5 cells (human fibroblast) were plated at appropriate dilutions and treated with AZD6244 (250 nM) or vehicle control. Cells were then exposed to graded doses of radiation and incubated for 10 to 14 days before counting of colonies.

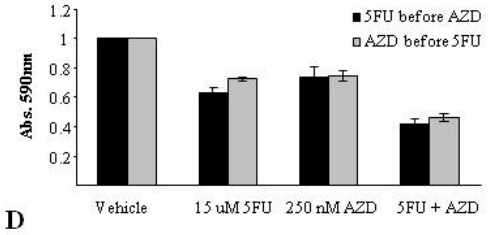
Recently, the effects of AZD6244 as a radiation modifier were extended to human colorectal carcinoma and lung xenografts.(41) We have recently extended our *in vitro* observations of enhanced radiation response with AZD6244 to the setting of chemoradiotherapy with 5-FU. We have found additional sensitization when 5-FU is added to AZD6244 in the setting of radiation suggesting a lack of interference with 5-FU. In addition, we found no evidence of a negative interaction between AZD6244 and 5-FU (Figure 3, unpublished data), an important consideration when considering the systemic therapeutic effects of Capecitabine. Additional work exploring the interaction of 5-FU and AZD6244 has shown that exposure to AZD6244 and 5-FU does not result in antagonism *in vitro* regardless of sequencing (unpublished data, data not shown).







%Proliferation Normalized to Vehicle Control		
Treatment	**5FU before AZD**	**AZD before 5FU**
100nM AZD6244	60.6 ± 3.8	58.2 ± 4.1
15uM 5FU	52.8 ± 6.1	45.4 ± 4.4
AZD + 5FU	38.9 ± 5.6	38.0 ± 3.9



%Proliferation Normalized to Vehicle Control		
Treatment	**5FU before AZD**	**AZD before 5FU**
250nM AZD6244	62.9 ± 3.5	72.3 ± 1.5
15uM 5FU	73.5 ± 7.3	74.6 ± 3.6
AZD + 5FU	42.1 ± 3.4	46.4 ± 2.5

Figure 3. Effects of AZD6244 on HCT 116 and HT29 cells. HT29 (A) and HCT116 (B) cells were plated at appropriate dilutions and treated with the indicated concentration of 5-fluorouracil (16 hour exposure prior to RT), AZD6244 (1 hour prior to RT), both AZD6244 and 5-FU, or vehicle control. Cells were then exposed to graded doses of radiation and incubated for 10 to 14 days before counting of colonies. After a similar treatment with no radiation, cells were incubated for 48 hours and an MTT assay was performed to assess proliferation in HT29 (C) and HCT116 (D) cells (5FU before AZD). A second set of MTT assays was performed to verify that treatment with AZD6244 prior to 5FU did not result in antagonism (AZD before 5FU).

We have demonstrated that inhibition of AZD6244 sensitizes to radiation induced tumor cell death via abrogation of the G2 checkpoint and an increase in mitotic catastrophe. Given the importance of signal transduction in radiation pro-survival mechanisms and the role of radiation in the standard management of locally advanced adenocarcinoma of the rectum, the addition of AZD6244 to radiation and Capecitabine is of interest.

2.5 **Correlative Studies Background**

The laboratory correlates will be performed with exploratory intent, using primarily descriptive techniques. Two classes of markers will be studied in blood and tumor tissues: 1) proteins expected to have altered phosphorylation or expression with AZD6244 treatment *and* 2) mutations that may predict for AZD6244 efficacy. Tumor tissue will be sampled prior to treatment, after a one week lead in phase of AZD6244 (day 8), and after one week of AZD6244 in combination with Capecitabine and radiation (day 15). Blood will be sampled for correlative studies prior to treatment, after a 7 day lead in phase of AZD6244, after one week of AZD6244 in combination with Capecitabine and radiation, and at one month following completion of all treatment. The markers that will be evaluated in blood include phosphorylation of ERK in peripheral blood mononuclear cells and levels of plasma TGFα.

2.5.1 Evaluation of mutations in *KRAS, BRAF, and PTEN* in tumor tissue.

Activating mutations in *KRAS* and B*RAF* are thought to predict for single agent efficacy with MEK inhibition(42) and may also be implicated in radiation response with AZD6244. This will be an exploratory endpoint for this study as the number of patients included are small and we estimate that only 40% of participants will have activating mutations. PTEN status may predict for resistance to MEK inhibition.(43) We hypothesize that the presence of activation mutations in *KRAS* or *BRAF* will be positively correlated with pathologic CR rate while loss of PTEN will be negatively correlated with pathologic CR. The presence of activating mutations will be evaluated via DNA purification, amplification via polymerase chain reaction, and sequencing. For *KRAS*, codons 12 and 13 will be amplified. For *BRAF*, exon 15 will be amplified. This analysis will be performed on pretreatment specimens only. These assays will be performed by the laboratory of pathology, NCI. If sufficient additional tissue or DNA remains, PTEN exon 1-9 amplification will be completed in the laboratory of Dr. Deborah Citrin followed by sequencing. This will be completed for tumors in which PTEN expression is not detected by immunohistochemistry.

2.5.2 Evaluation of tumor ERK phosphorylation and PTEN expression

ERK is the immediate downstream target of MEK and may be phosphorylated at baseline or after radiation. Comparisons of pre and post treatment changes with immunohistochemistry may provide direct evidence of AZD6244 effect in tumors. In addition, tumor ERK phosphorylation has not yet been correlated to PBMC ERK phosphorylation in humans. To provide evidence that PBMC ERK phosphorylation is an accurate surrogate for tumor ERK phosphorylation, we plan to evaluate these markers in the patients enrolled on this trial at three time points: 1) prior to treatment, 2) after a one week lead in of AZD6244, and 3) after one week of combined AZD6244, Capecitabine, and radiation. We have shown that radiation and 5-FU as single agents or in combination induce ERK phosphorylation in colorectal cell lines (Figure 4). We hypothesize that treatment with AZD6244 will result in reduced ERK phosphorylation in PBMC and tumor tissue after single agent treatment with AZD6244 and after combined treatment.

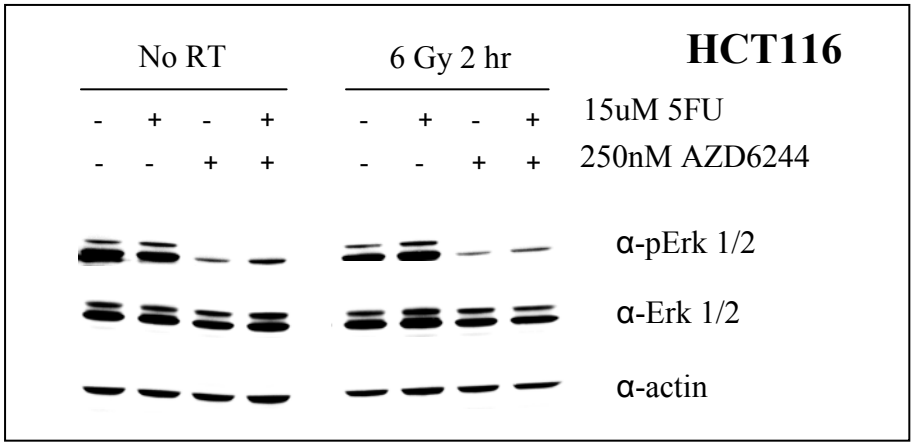


Figure 4. Effects of AZD6244 on ERK phosphorylation after treatment with radiation (RT), 5-FU, and/or AZD6244. Cells were plated and treated with AZD6244, 5-FU, and RT at the doses indicated in a fashion identical to that performed for clonogenic assays. Cells were harvested and subjected to western blotting two hours after IR.

If sufficient tissue is available, additional staining for PTEN expression will be performed on initial biopsies. In tumors with absent PTEN expression, the presence of PTEN mutations will be assessed. PTEN status may predict for resistance to MEK inhibition.(43)

2.5.3 Evaluation of PBMC ERK phosphorylation

The ability to detect changes in PBMC following treatment with AZD6244 has been demonstrated in Phase I trials (ARRY-0401), however this has not to our knowledge been correlated to tumor biopsies. We hope to determine if a correlation exists between alterations in circulating lymphocyte pERK and tumor pERK during AZD6244 treatment. Whole blood will be collected at the same time points as tumor biopsies: 1) prior to treatment, 2) after a one week lead in of AZD6244, and 3) after one week of combined AZD6244, Capecitabine, and radiation. ERK phosphorylation will also be obtained at the times of pharmacokinetic (PK) sampling on day 4 or 5 and day 11 or 12. See section 2.5.5 for a discussion of PK sampling time points and analysis.

2.5.4 Evaluation of plasma TGF alpha

Data generated in our laboratory supports that the radiation sensitizing effects of AZD6244 are in part related to a reduction in TGFα secretion with AZD6244 treatment. TGFα is secreted after radiation and is thought to act as a survival factor through EGFR-Akt signaling. We have shown that AZD6244 treatment reduces TGFα secretion at baseline and after irradiation (figure 5, unpublished data).

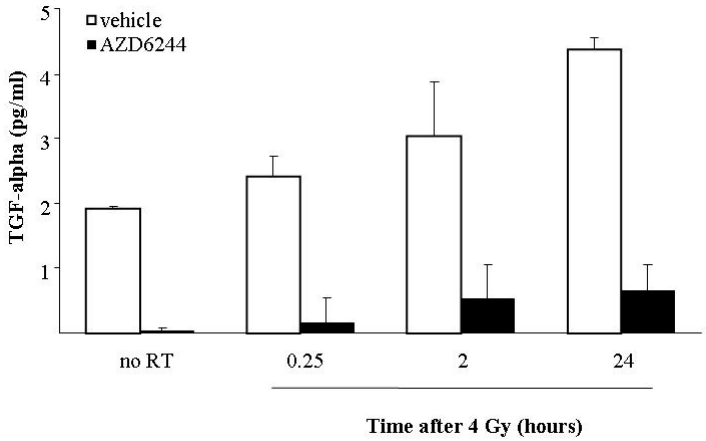


Figure 5. The effects of AZD6244 on radiation induced TGF-α secretion in A549 cells. A549 cells were exposed to AZD6244 (250 nM) or vehicle control for 16 hours, irradiated, and incubated until the time points shown. Media was collected and subjected to ELISA for soluble TGF-α. TGF-α secretion was induced by irradiation in a time dependent fashion. Basal and radiation-induced TGF-α secretion was dramatically reduced with AZD6244 treatment.

We have also shown that inhibition of TGFα with a blocking antibody sensitizes A549 cells to radiation (unpublished data). Supplementation of TGFα in the media of cells treated with AZD6244 resulted in a reduction in radiation sensitization and a reduction in the number of cells undergoing mitotic catastrophe (figure 6, unpublished data). These results suggest that TGFα is a survival factor after radiation that is reduced with AZD6244. Therefore, a comparison of baseline and post-treatment TGFα may provide a marker of radiation sensitization with AZD6244.

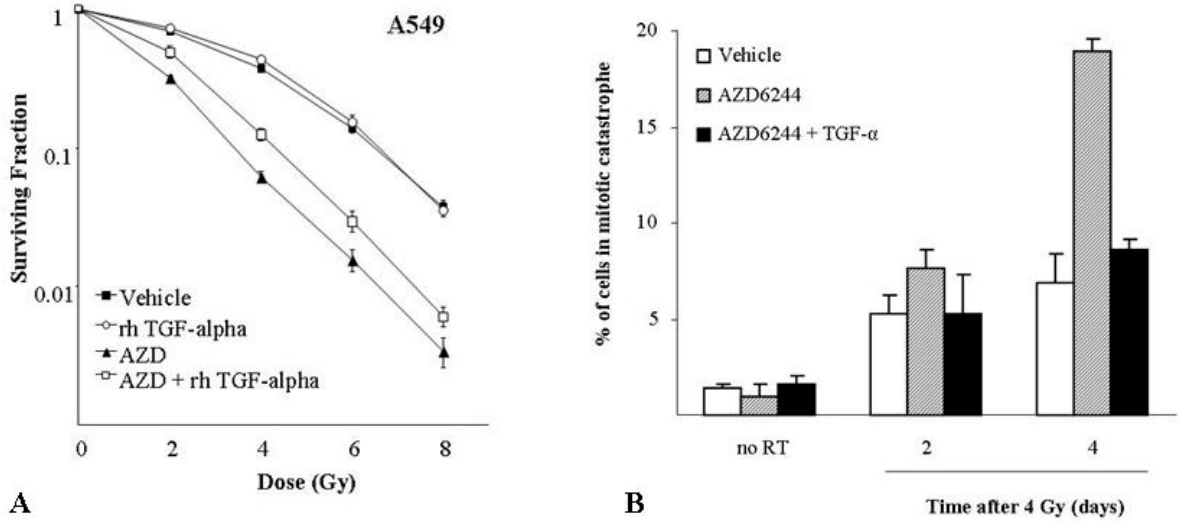


Figure 6. The effects of TGF-α on cell survival after irradiation and treatment with AZD6244. A) A549 cells were exposed to AZD6244 (250 nM) or vehicle control for 16 hours, and irradiated with graded doses of X-rays. Recombinant human TGF-α (5 pg/ml) was supplemented in media at the time of irradiation. Colony-forming efficiency was determined 14 days later and survival curves generated after normalizing for cell killing by AZD6244 alone. There was no measurable increase or decrease in plating efficiency with the addition of recombinant human TGF-α in the absence of irradiation. The data represent the mean of three independent

experiments. B) A549 cells growing in chamber slides were exposed to AZD6244 (250 nM) or vehicle control, irradiated (4 Gy), and fixed at the specified times evaluation of mitotic catastrophe. Nuclear fragmentation was evaluated in 150 cells per treatment per experiment. Nuclear fragmentation was defined as the presence of two or more distinct lobes within a single cell. PE, plating efficiency with AZD6244; DEF, dose enhancement factor. Columns, mean; bars, SE. Nuclear fragmentation was defined as the presence of two or more distinct lobes within a single cell.

We have confirmed that changes in tumor levels of TGFα can be observed in tumors in vivo (Figure 7). Plasma TGFα has been evaluated as a molecular marker of response in a recent clinical trial of chemoradiation with cetuximab for treatment of patients with rectal cancer.(44) In this study TGFα was shown to be altered after treatment with cetuximab. We hypothesize that a reduction in TGFα will be observed in the plasma of patients treated with AZD6244. The use of TGFα as a marker with MEK inhibition may prove more useful compared to EGFR inhibition (cetuximab) due to the high rate of Ras mutations in colorectal tumors. The usefulness of TGFα levels for response to EGFR inhibition may be questionable because TGFα is induced by ERK phosphorylation which should be constitutively activated in the setting of Ras mutations, regardless of the activation status of the EGF receptor. Because MEK, the target of AZD6244 is downstream of Ras, it is possible that TGFα will be a much more informative marker in the setting of Ras mutations.

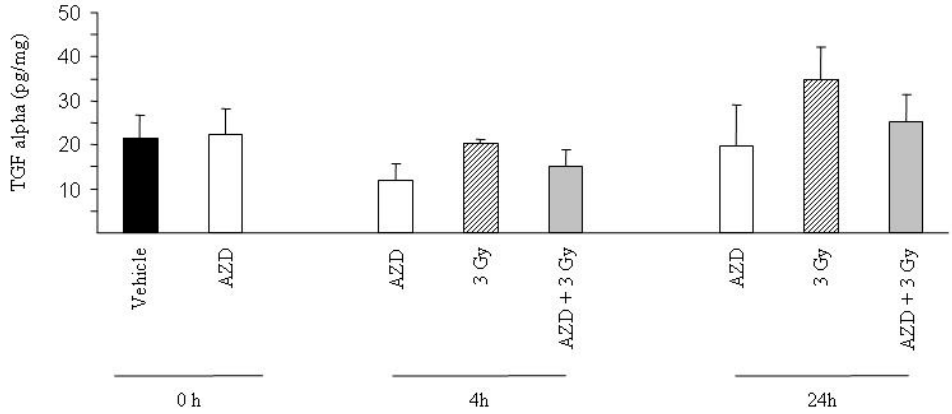


Figure 7. The effects of AZD6244 and radiation on TGF alpha levels in A549 xenografts. When flank A549 xenograft tumors reached 177 mm3 in size, mice were randomized into four groups: vehicle, AZD6244, radiation, or AZD6244 plus radiation. AZD6244 was dissolved in water and given by oral gavage at 50 mg/kg delivered in a single dose. Radiation (3 Gy) was delivered 4 hours after the AZD6244 treatment. Each treated group contained 3 mice. Tumors were harvested at the time points shown and homogenized. Lysates were subjected to ELISA for soluble TGF-α and values were normalized to tumor weight. TGF-α levels were induced by irradiation and reduced with AZD6244 treatment. AZD: AZD6244. Columns, mean; bars, SE

2.5.5 Pharmakokinetic Measurements

Blood samples (2 ml) for the determination of plasma AZD6244, N-desmethyl AZD6244 and AZD6244 Amide determination will be collected from patients in a comprehensive PK sampling schedule. Blood will be immediately placed on ice and the specimen transported to the laboratory of Deborah Citrin for processing and storage. Analysis of plasma samples for the determination of AZD6244, N-desmethyl AZD6244 and AZD6244 Amide concentrations will be the responsibility of the Clinical Pharmacology

& DMPK(Drug Metabolism and Pharmacokinetics) Department, Alderley Park, AstraZeneca, UK. Specimens will be shipped in batches from Dr. Deborah Citrin's laboratory to Astra Zeneca's contract laboratory for analysis.

For once daily dosing (dose level -1)

- Day 4 or 5 (AZD6244 alone): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8, 12 and 24 hours post-dose (24 hour sample is pre- the next day's dose).
- Day 11 or 12 (AZD6244 + capecitabine + radiotherapy): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8, 12 and 24 hours post-dose (24 hour sample is pre- the next day's dose).

For twice daily dosing (dose levels 1 and 2):

- Day 4 or 5 (AZD6244 alone): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8 and 12 hours post-dose (12 hour sample is pre- the next day's dose).
- Day 11 or 12 (AZD6244 + capecitabine + radiotherapy): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8 and 12 hours post-dose (12 hour sample is pre- the next day's dose).

The actual sample time and date of all PK samples will be recorded.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed locally advanced, non-metastatic adenocarcinoma of the rectum (clinical stage T3 anyN, T4 anyN, or AnyT N+).

3.1.2 Patients with recurrent adenocarcinoma of the rectum with no clinically evident distant disease will be eligible if they are deemed to have pelvic nodal metastases or disease extending through the muscularis of the rectum. These patients should be evaluated by a Radiation Oncologist, Medical Oncologist and Surgeon prior to enrolling on study to confirm anticipated resectability. These patients should not have received prior radiotherapy for management of their rectal cancer.

3.1.3 Age $\geq$18 years.

3.1.4 ECOG performance status $\leq$1 (Karnofsky $\geq$70%, see Appendix A).

3.1.5 Patients must have normal organ and marrow function as defined below:

- leukocytes $\geq$3,000/mcL
- absolute neutrophil count $\geq$1,500/mcL
- platelets: $\geq$100,000/mcL

– total bilirubin:	within normal institutional limits except for patients with Gilbert's who must have a direct bilirubin < 1.0 mg/dL
– AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal
– creatinine	within normal institutional limits OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.6 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for six months after the completion of radiation. Women of child-bearing potential must have a negative pregnancy test prior to entry. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Adequate contraception for male patients should be used for 6 months after irradiation.

3.1.7 Ability to understand and the willingness to sign a written informed consent document.

3.1.8 Willingness to sign a release of medical records pertaining to previous and future treatment for rectal cancer.

3.2 Exclusion Criteria

3.2.1 Chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or lack of recovery from adverse events due to agents administered more than 4 weeks earlier.

3.2.2 Patients may not be receiving any other investigational agents.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to AZD6244 or other agents used in study.

3.2.4 Previous MEK inhibitor use.

3.2.5 Contraindications to radiotherapy to the pelvis such as inflammatory bowel disease or known genetic sensitivity to ionizing radiation such as ataxia telengiectasia.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Patients with QTc interval >470 msecs or other factors that increase the risk of QT prolongation or arrhythmic events (*e.g.*, heart failure, uncorrectable hypokalemia, family history of long QT interval syndrome) including heart failure that meets New York Heart Association (NYHA) class III and IV definitions (see Appendix B) are excluded.

3.2.8 Required use of a concomitant medication that can prolong the QT interval. See Appendix C for a table of medications with the potential to prolong the QTc interval. A comprehensive list of agents with the potential to cause QTc prolongation can be found at http://www.arizonacert.org/medical-pros/drug-lists/drug-lists.htm.

3.2.9 Refractory nausea and vomiting, chronic gastrointestinal diseases (e.g. inflammatory bowel disease), or significant bowel resection that would preclude adequate absorption.

3.2.10 HIV-positive patients on combination antiretroviral therapy.

3.2.11 Known dihydropyrimidine dehydrogenase deficiency.

3.2.12 History of prior radiation to the pelvis

3.2.13 For patients with newly diagnosed rectal cancer, prior therapy for adenocarcinoma of the rectum with the exception of diverting colostomy if required to relieve obstruction (including chemotherapy).

3.2.14. Patients with recurrent rectal cancer may not have undergone prior radiotherapy for rectal adenocarcinoma or have received therapy for the recurrence with the exception of diverting colostomy if required to relieve obstruction.

3.2.15 History of myocardial infarction within the past 6 months or history of ventricular arrhythmia

3.2.16 Uncontrolled hypertension

3.2.17 Pregnant or lactating females are excluded

3.3 Research Eligibility Evaluation

All referred patients who meet the eligibility criteria will be evaluated in the Radiation Oncology Clinic at the National Cancer Institute. All screening studies will be performed within 14 days of protocol enrollment unless otherwise stated.

3.3.1 Clinical evaluation including documentation of performance status, medications and prior history of radiation, including smoking history

- EKG for determination of QTc
- Total colonoscopy within 8 weeks of onset of study when possible. This may be performed at an outside institution as long as the report is available for

review.
- Pulse oximetry
- Echocardiogram or MUGA

3.3.2 Radiographic studies:
- CT (chest, abdomen and pelvis) to confirm the absence of metastases will be reviewed to confirm the presence of resectable disease within 4 weeks of onset of study (outside studies may be used if acquired in the appropriate timeframe).
- Transrectal ultrasound or MRI of the pelvis to stage primary or locally recurrent rectal tumor within 4 weeks of onset of study (outside studies may be used if acquired in the appropriate timeframe).

3.3.3 Laboratory studies:
- Serum creatinine, Serum hepatic panel, complete blood count with differential, APTT, PT. Urine pregnancy test for women of childbearing potential, serum CEA level

3.3.4 Pathologic assessment:
- A copy of path report along with slides and blocks of biopsy material for review by NCI Surgical Pathology.

3.3.5 Surgical consultation:
- NIH surgical consultation will be obtained for all patients.

3.4 Registration Procedures

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the Web site (http://camp.nci.nih.gov/ccr/welcome.htm) must be completed and faxed to (301) 480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail. Please note that it is very important for all registrars to acquire encrypted e-mail from the NIH Help Desk since the verification of registration includes patient information. A recorder is available during non-working hours.

4. TREATMENT PLAN

4.1 Protocol Therapy Administration

4.1.1 AZD6244 administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for AZD6244, radiation, and Capecitabine are described in Section 6. Appropriate dose modifications for AZD6244 and Capecitabine are described in Section 5. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Dose Escalation Schedule			
Dose Level	**Dose**		
	AZD6244	**Capecitabine**	**Radiation***
Level -1	50 mg PO QD Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
Level 1	50 mg PO BID Weeks 1-7	825 mg/m^2 PO e every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
Level 2	75 mg PO BID Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
* An additional 360 cGy may be delivered for fixed T4 tumors for a total dose of 5400 cGy			

Dose-escalation of AZD6244 will begin at dose level 1, 50 mg PO BID delivered continuously, 7 days per week beginning one week prior to the initiation of chemoradiotherapy and continuing until the last day of chemoradiotherapy. Dose level -1 will only be delivered if Dose Level 1 is considered to be not tolerated due to toxicity observed in dose level 1 patients.

- Patients will be provided with a Medication Diary for AZD6244 (Appendix D), instructed in its use, and asked to bring the diary with them to each appointment.
- Take AZD6244 capsules on an empty stomach (either 1 hour before or 2 hours after meals). AZD6244 capsules should be taken with water only.
- In the event of AZD6244 de-escalation to dose level -1 (50 mg PO QD), the patient will be instructed to take AZD6244 as a morning dose.
- Follow up for certain adverse events (AEs) should be performed to better characterize the effects of AZD6244 therapy:
    - Patients experiencing **edema** should have cardiac ejection fraction (EF) measurements, electrocardiogram, serum chemistry (including electrolytes and albumin), and routine urinalysis.
    - Patients with symptoms consistent with **cardiac** impairment (*e.g.,* congestive cardiac failure, edema, or dyspnea) should have EF measurements (MUGA scan or echocardiography) and electrocardiogram at the time of the event as well as other routine investigations.
    - **Respiratory** events (including dyspnea or pulmonary edema) should be followed up with an electrocardiogram, EF measurement, and chest X-ray.
    - Oxygen saturation will be measured at baseline and again following any **respiratory event**.
    - Patients experiencing **visual disturbances** should undergo a complete ophthalmologic examination, including a slit lamp examination.

4.1.2 Capecitabine administration

Capecitabine will be administered at a dose of 825 mg/m^2 PO every 12 hours delivered approximately two hours prior to radiotherapy and the second dose will be delivered 12 hours later. Doses will be rounded to the nearest 150 mg. Patients will begin Capecitabine dosing on the first day of radiotherapy and will continue to receive Capecitabine on days of radiotherapy (Monday through Friday) until the final day of radiotherapy. Patients will be instructed to take Capecitabine with food.

Administration with Warfarin can increase the INR beyond expected. Capecitabine should not be administered with aluminum hydroxide or magnesium hydroxide containing antacids, such as Maalox, as it may increase the plasma concentrations of capecitabine and its metabolites.

4.1.3 Radiotherapy administration

4.1.3.1 Modality, Fractionation, and total dose

Radiation will be delivered with external beam radiation with megavoltage radiation with beam energies of 6MV or higher. Treatment will be delivered in 1.8 Gy fractions 5 days per week with every field treated each day. The total dose to the PTV encompassing the pelvic lymph nodes and tumor will be 45 Gy in 25 fractions. A boost to the tumor will be delivered for an additional 5.4 Gy in 1.8 Gy fractions. Fixed T4 tumors may receive a boost of 9 Gy for a total dose of 54 Gy.

4.1.3.2 Simulation

Patients will be simulated supine or prone as appropriate to the clinical status and staging. Simulation will be performed with a CT simulator in the Radiation Oncology Branch. Oral contrast will be delivered approximately one hour prior to simulation to allow opacification of small bowel unless contraindicated. Rectal contrast will be delivered just prior to simulation unless contraindicated. An anal marker will be used at the time of simulation.

4.1.3.3 Volume definitions

Initial pelvic field: The gross tumor volume (GTV) will be defined as all gross disease evident on imaging and examination. The clinical target volume (CTV) will be defined as the combination of
1) the rectal GTV with a 2.5 cm craniocaudal margin and a 1.5 cm radial margin
2) the nodal GTV with 1.5 cm margin
3) the iliac vessels with 1 cm margin (including the external iliac vessels only if T4 and invading anterior structures) extending craniocaudally to the L5/S1 interspace
4) the presacral space from S1-S5
5) the mesorectal and perirectal lymphatics. The planning target volume will be defined as the CTV with a 5-7 mm margin.

Boost field: The boost field will include the gross tumor including involved lymph nodes with a 2.0 cm margin to CTV. The boost field CTV must include the sacral hollow. The PTV will be defined as CTV with 5 to 7 mm margin.

4.1.3.4 Dose specification

The PTV doses should meet the following criteria:
1) > 93% of the PTV should receive at least 93% of the prescribed dose
2) < 5% of the PTV should receive more than 110% of the prescribed dose. Efforts will be made to reduce heterogeneity if possible.

4.1.3.5 Normal structures

Minor modifications to normal tissue dose constraints will be made if the total tumor dose is 5400 cGy. Every effort will be made to minimize areas of non target tissue that receive target doses. Specific attention will be paid to reducing exposure to small bowel, ileocecal region, femoral neck, bladder, and anal sphincter if uninvolved. The following organs will be contoured for treatment planning purposes and will be evaluated with dose volume histograms: bladder, femoral neck and head, small bowel.

The following dose goals will apply for normal tissues:

Small bowel: No more than 180 cc above 35 Gy, No more than 100 cc above 40 Gy, No more than 65 cc above 45 Gy, No small bowel volume should receive 50 Gy

Femoral heads: No more than 40% volume above 40 Gy, No more than 25% volume above 45 Gy, No femoral head volume should receive 50 Gy

Bladder: No more than 40% volume above 40 Gy, No more than 15% volume above 45 Gy, No bladder volume should receive 50 Gy

4.1.4 Surgery

Surgery is not part of this protocol. It is anticipated that at the completion of chemoradiation patients will have a four to six week recovery period and will undergo surgical resection as is the current clinical standard. Surgical specimens will be reviewed by the Surgical Pathology laboratory at NIH for examination and determination of response.

4.2 Definition of Dose-Limiting Toxicity

- Dose-limiting toxicities will be defined as follows (during treatment and within the first three weeks after treatment):
  1. Recurring and persistent Grade 3 diarrhea despite appropriate medical therapy.
  2. ANC <500 for more than 5 days or neutropenic fever
  3. Grade 4 thrombocytopenia
  4. Recurring and persistent Grade 3 nausea/vomiting despite appropriate anti-emetic therapy
  5. Grade 4 dermatitis acneiform rash
  6. Grade 4 fatigue
  7. All other non-hematologic toxicities of grade 3 or higher except electrolyte abnormalities that are correctable
  8. Delays in radiation treatment due to toxicity of more than 2 weeks (cumulative duration over the course of the entire treatment).

- Management and dose modifications associated with the above adverse events are outlined in Section 5.

- Dose escalation will proceed within each cohort according to the following scheme. Dose-limiting toxicity (DLT) is defined above.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
$\geq$2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. If $\geq$2 initial patients experience a DLT at dose level 1, de-escalation to dose level -1 will occur.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

4.3 **General Concomitant Medication and Supportive Care Guidelines**

Because there is a potential for interaction of AZD6244 and Capecitabine with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes (2C9 and 1A2).

- Investigators should document the patient's comedications to investigate any potential drug interactions.
- Because there is a potential for interaction of AZD6244 with other concomitantly administered drugs through the cytochrome P1A2 system, the case report form (CRF) must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. Throughout the study, patients should avoid changes to, or the addition of all concomitant medications, in particular any that may affect the metabolism of AZD6244 (eg, CYP1A2 or 3A4 inhibitors/inducers), unless considered clinically indicated. If patients are taking strong inducers or inhibitors of CYP1A2 these medications will be discontinued prior to initiation of protocol therapy.
- Because there is a potential for interaction of Capecitabine with other concomitantly administered drugs through the CYP2C9 system, the case report form (CRF) must capture the concurrent use of all other drugs, over-the-counter

medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to interact with Capecitabine. Patients will be transitioned to an acceptable alternative if available. Common examples include aluminum and magnesium hydroxide containing antacids, coumadin, and phenytoin.

- Diarrhea will be managed with the following regimen: loperamide (4 mg PO) at onset of symptoms, followed by 2 mg loperamide every 2 hours while awake (or 4 mg PO every 4 hours while sleeping) up to a maximum of 16 mg loperamide per day. Additional agents may be used concurrently if loperamide is not adequate to control diarrhea as a single agent.
- The use of white cell growth factors is discouraged during the course of radiation but is allowed for management of neutropenia at physician discretion in concordance with ASCO guidelines.
- Nausea and vomiting will be managed through the use of appropriate simple supportive measures (*e.g.,* prochlorperazine). To date, 5 HT3 antagonists have not been routinely required but allowed if necessary.
- Patients will receive full supportive care as needed while on this study including blood product support, intravenous hydration, antibiotic treatment and treatment of other newly diagnosed or concurrent medical conditions.
- Patients should be advised to drink plenty of water or take rehydration fluids to avoid dehydration if diarrhea occurs.

4.4 **Duration of Therapy**

Treatment with AZD6244 will continue until the completion of chemoradiotherapy or until one of the following criteria applies:

- Toxicity requiring discontinuation (section 4.2)
- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

In the event of a discontinuation of AZD6244 for a dose limiting toxicity the patient will continue to receive capecitabine and radiotherapy if not already completed.

4.5 **Duration of Follow Up**

Patients will be followed for adverse events until three weeks after completion of chemoradiation. After this time they will be followed for the purpose of describing RTOG late toxicities until 36 months.

4.6 **Criteria for Removal from Study**

Patients will be removed from study after follow up has been completed as in section 4.5 or if

- The patient refuses further follow up care at NIH

- General or specific changes in the patient's condition render the patient unacceptable for follow up in the judgment of the investigator
- Death.

The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.0 DOSING DELAYS/DOSE MODIFICATIONS

5.1 **AZD6244**

AZD6244 dose reductions will occur in the event of a DLT. In the event of a DLT that is an overlapping toxicity with Capecitabine, AZD6244 will be dose reduced. If a Capecitabine associated toxicities occur following discontinuation of AZD6244, Capecitabine dose adjustments will occur as deemed appropriate by the Principal Investigator in conjunction with the participating Medical Oncology Associate Investigator. AZD6244 dose reductions will not occur for established non-DLT Capecitabine toxicities (e.g. Grade 2 hand-foot syndrome).

Dose de-escalation will occur as described in the table below for DLT's and the following overlapping toxicities

- **Grade ≥3 diarrhea that persists despite appropriate medical management including anti-diarrheal medications**
- **Grade ≥3 nausea and vomiting that persists despite appropriate medical management including anti-nausea medications**
- **Grade 2 hand foot that persists despite interruption of capecitabine**

Dose De-Escalation Schedule			
Dose Level	**Dose**		
	AZD6244	**Capecitabine**	**Radiation***
Level -1	50 mg PO QD Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
Level 1	50 mg PO BID Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
Level 2	75 mg PO BID Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
* An additional 360 cGy may be delivered for fixed T4 tumors for a total dose of 5400 cGy			

Diarrhea must be managed symptomatically. IV hydration and use of loperamide, as well as close observation, are recommended for diarrhea if clinically indicated. C If control takes

longer than 2 days, medical evaluation including relevant diagnostic procedures, alternative treatment, and possible investigation of DPD deficiency should be considered. Capecitabine and AZD6244 may be held for up to 72 hours and will not be re-started until diarrhea has resolved to grade ≤1. In patients with grade 3 diarrhea evaluation for C. difficile colitis should be considered. Patients with neutropenia and diarrhea should be considered for empiric use of prophylactic antibiotics such as oral quinolones.

5.2 **Capecitabine**

Capecitabine will be withheld in cases of Grade 3 or greater neutropenia or Grade 2 or greater hand-foot syndrome or mucositis that do not respond to medical management. Capecitabine will be restarted after the symptoms improved to Grade 1. The dose of Capecitabine may be adjusted in the event of recurrent Grade 2 or greater hand-foot syndrome, neutropenia, or other Capecitabine toxicities that have not been attributed to AZD6244.

Summary of management of common Capecitabine toxicities during combined AZD6244 and Capecitabine dosing	
Toxicity	**Capecitabine dose modification**
Grade 2 hand foot syndrome	Interrupt until ≤ grade 1. May then restart capecitabine at full dose. For second occurrence, hold capecitabine until ≤ grade 1, then restart capecitabine one dose level lower (75% of starting dose first occurrence, 50% of the starting dose the second occurrence, discontinue the third occurrence).
Grade 3 hand foot syndrome	Interrupt until ≤ grade 1. Then restart capecitabine one dose level lower (75% of starting dose first occurrence, 50% of the starting dose the second occurrence, discontinue the third occurrence).
Grade 2 thrombocytopenia	Continue at current dose
Grade 3 thrombocytopenia	Hold until recovery until ≤ grade 1 then resume one dose level lower*
Grade 4 thrombocytopenia	Hold until recovery until ≤ grade 1 then resume one dose level lower*
Grade 3 neutropenia	Hold until recovery until ≤ grade 1 then resume one dose level lower*
Grade 4 neutropenia	Hold until recovery until ≤ grade 1 then resume one dose level lower*
≥ Grade 3 febrile neutropenia	Hold until resolution of fever and neutropenia to ≤ grade 1. Then resume on dose level lower*
*Dose reductions for capecitabine 1. Starting dose 2. 75% of the starting dose 3. 50% of the starting dose	

4. Discontinuation of capecitabine.

5.3 **Radiation**

Radiation dose will not be adjusted based on toxicity. Delay in treatment may occur in the event that it is felt to be unsafe to deliver treatments due to the clinical status of the patient. Radiation treatment delays will be minimized as efficacy of radiotherapy is decreased with treatment delay. In the event that radiation is delayed for a toxicity, treatment with AZD6244 and capecitabine will be held until resumption of radiation. AZD6244 and capecitabine dose will be modified as described in section 5.1 and 5.2.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 6.2) and the characteristics of an observed AE (Section 6.3) will determine whether the event requires expedited (via AdEERS) **in addition** to routine reporting.

6.1 **Definitions**

6.1.1 **Adverse Event**

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted above in Section 6.1.
All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

6.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

6.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

6.1.4 Serious

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

6.1.5 Disability

A substantial disruption of a person's ability to conduct normal life functions.

6.1.6 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

6.1.7 Protocol Deviation (NIH Definition)

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the IRB.

6.1.8 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the study procedures in an IRB-approved research protocol that has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

6.1.9 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

(a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
(b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.2 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

6.2.1 CAEPRs for CTEP-Supplied Investigational Agent(s)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single, complete list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (the ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" (http://ctep.cancer.gov/reporting/adeers.html) for further clarification. The CAEPR may not provide frequency data; if not, refer to the Investigator's Brochure for this information.

6.2.1.1 CAEPR for AZD6244

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for

Selumetinib (AZD6244 Free base [NSC 741078], AZD6244 Hydrogen sulfate [NSC 748727])

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via AdEERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 665 patients.* Below is the CAEPR for Selumetinib (AZD6244).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.3, September 27, 2011[1]

Adverse Events with Possible Relationship to Selumetinib (AZD6244) (CTCAE 4.0 Term) [n= 665]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
		Febrile neutropenia[2]	
CARDIAC DISORDERS			
		Left ventricular systolic dysfunction	***Left ventricular systolic dysfunction (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
Diarrhea[3]			***Diarrhea[3] (Gr 3)***
	Dry mouth		
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema face		***Edema face (Gr 2)***
Edema limbs			***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 3)***
	Platelet count decreased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Hypoalbuminemia		
	Hypomagnesemia		

NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		***Headache (Gr 2)***
PSYCHIATRIC DISORDERS			
	Insomnia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Dry skin		***Dry skin (Gr 2)***
	Pruritus		
Rash acneiform			***Rash acneiform (Gr 3)***
Rash maculo-papular			***Rash maculo-papular (Gr 2)***
VASCULAR DISORDERS			
	Hypertension		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV . Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Febrile neutropenia/neutropenic infection has been observed primarily in trials combining Selumetinib (AZD6244) and docetaxel.

[3]SBE-CD (Captisol®, vehicle) in the mix and drink formulation is known to cause soft stools and/or diarrhea in rats and dogs; however, it is possible that some of these findings might be related to exacerbation of the vehicle effect by Selumetinib (AZD6244).

[4]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on Selumetinib (AZD6244) trials but with the relationship to Selumetinib (AZD6244) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (hemorrhagic anemia)
CARDIAC DISORDERS - Acute coronary syndrome; Cardiac disorders - Other (Takatsubo cardiomyopathy syndrome); Chest pain - cardiac; Heart failure; Palpitations; Right ventricular dysfunction; Sinus bradycardia
EYE DISORDERS - Blurred vision; Extraocular muscle paresis; Eye disorders - Other (bilateral macular edema); Eye disorders - Other (black haze in line of vision); Eye disorders - Other (elevated intraocular pressure); Flashing lights; Glaucoma; Retinopathy
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Colitis; Dyspepsia; Esophagitis; Flatulence; Gastric hemorrhage; Gastroesophageal reflux disease; Ileal stenosis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure; Hepatobiliary disorders - Other (cholangitis)
INFECTIONS AND INFESTATIONS - Infection[4]
INVESTIGATIONS - CPK increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged; Neutrophil count decreased; Weight gain
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypokalemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (joint swelling); Musculoskeletal and connective tissue disorder - Other (rhabdomyolysis); Myalgia; Myositis; Pain in extremity

NERVOUS SYSTEM DISORDERS - Cognitive disturbance; Depressed level of consciousness; Dysgeusia; Dysphasia; Ischemia cerebrovascular; Leukoencephalopathy; Memory impairment; Nervous system disorders - Other (numbness); Nervous system disorders - Other (spinal cord compression); Oculomotor nerve disorder; Peripheral sensory neuropathy; Reversible posterior leukoencephalopathy syndrome; Seizure
PSYCHIATRIC DISORDERS - Confusion; Depression
RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Epistaxis; Hypoxia; Pharyngolaryngeal pain; Pleural effusion; Pneumonitis; Sore throat; Voice alteration
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Scalp pain; Skin and subcutaneous tissue disorders - Other (angular cheilitis, unilateral); Skin and subcutaneous tissue disorders - Other (skin fissures)
VASCULAR DISORDERS - Hypotension; Thromboembolic event

Note: Selumetinib (AZD6244) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

6.2.1.2 Adverse Event List for Capecitabine

Capecitabine may cause diarrhea, sometimes severe. Standard anti-diarrheal treatments are recommended and dose reductions are employed for reoccurrence of grade 3 diarrhea. Grade 3 or 4 toxicities are described below for patients receiving Capecitabine as a single agent: diarrhea (12%), nausea (2%), stomatitis (2%), vomiting (2%), abdominal pain (3%), hand- and –foot syndrome (17%), decreased neutrophils/granulocytes (2.4%), hyperbilirubinemia (20%), decreased lymphocytes (13%). A comprehensive list of adverse events observed with Capecitabine can be found in the package insert. Diarrhea and rash are overlapping toxicities of AZD6244 and capecitabine and will be aggressively managed as per section 5.1.

6.3 Adverse Event Characteristics

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

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 6.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 6.2.1.1).

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

- In addition, the study will use the RTOG/EORTC Radiation Morbidity Scoring Scheme to score side effects of radiation treatment for descriptive purposes. These criteria are available from the RTOG website http://rtog.org/members/toxicity/main.html

6.4 Expedited Adverse Event Reporting

6.4.1 Expedited AE reporting for this study must use AdEERS (Adverse Event Expedited Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined in the table below (Section 6.4.2).

In the rare event when Internet connectivity is disrupted a 24 hour-hour notification is to be made to NCI by telephone at: 301-897-7497 for CIP studies. An electronic report MUST be submitted immediately upon re-establishment of internent connection. Please note that all paper AdEERS forms have been removed from the CTEP website and will NO LONGER be accepted.

6.4.2 Expedited Reporting Guidelines – AdEERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 1 Trials

Phase 1 Trials								
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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Note: All deaths on study require both routine and expedited reporting

regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

6.4.3 Protocol-Specific Expedited Adverse Event Reporting Exclusions
Toxicities of surgery will not be collected as adverse events. Adverse events will be recorded and reported until three weeks after the completion of chemoradiotherapy for all patients. From 3 weeks following chemoradiation until 3 years of follow-up only the RTOG late toxicity scoring schema will be collected.

6.4.4 NCI-IRB Reporting

6.4.4.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

*The NCI-IRB **requires** that the following language be used for reporting Expedited Adverse Events to the NCI-IRB:*

The Protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research
- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems

Reports must be received by the NCI-IRB within 7 working days of PI awareness via

iRIS.

6.4.4.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

For reporting of adverse events at time of continuing review, the NCI-IRB requires a summary report of adverse events that have occurred on the protocol **since the previous continuing review and in aggregate**. The method of presentation should provide the NCI-IRB with the information necessary to clearly identify risks to participants and to make a risk: benefit determination. The summary report is based on the following guidance: any unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk: benefit of study participants in the narrative.

The following table is recommended for use of reporting:

CTCAE Term	Grade	# of Events since last CR	Total # of Events	Attribution to IND	Attribution to Research	Listed in Consent?

The protocol PI will report to the NCI-IRB:

1. All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
2. All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
3. All Grade 5 events regardless of attribution;
4. All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

6.4.4.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that require a sponsor recommended change to the protocol or the consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.
This is based on the January 15, 2007 OHRP Guidance "Guidance for Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events" under Section B. Reporting of external adverse events by investigators to IRBs: *http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm*

6.5 **IND Sponsor Reporting; Refer to 6.2 and 6.4**

An investigator must immediately report to the sponsor any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

6.6 **FDA Reporting Criteria**

6.6.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

6.6.1.1 Expedited Reporting to the FDA

The Sponsor will notify FDA via phone, fax, or email of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information. This will be followed with a written report within 15 days using the MedWatch Form 3500a.

The study Sponsor will notify FDA in writing of any suspected adverse reaction that is both serious and unexpected as soon as possible but no later than 15 calendar days after initial receipt of the information using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

The study Sponsor will also report expeditiously as above:

- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

6.6.1.2 Exclusions to expedited reporting to the FDA

The following events will not be reported in an expedited manner but will be included in the annual report:

6.6.1.3 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

6.7 **Secondary AML/MDS**

All secondary malignancies that occur following treatment will be reported through AdEERS, CTCAE v4.0.

6.8 **Data and Safety Monitoring Plan**

6.8.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis weekly when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations and violations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

6.8.2 Sponsor Monitoring Plan

Data reporting as per 12.1.

7. Human Subjects Protection

7.1 Rationale for Subject Selection

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women and children are excluded from this study. This study will be recruited through internal referral, our physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer.) Participants should realize that there is no guarantee of benefit to them from participation in this trial. The results of this trial may benefit future cancer patients. To date, there is no information that suggests that differences in drug metabolism or effect on tumor would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, a follow-up study may be written to investigate those differences more fully. Inclusion of Women and Minorities: This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met.

7.2 Justification for Exclusions

Pregnant women are excluded from this study because the effects of AZD6244 on the developing fetus is unknown and because radiation is a known teratogen. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with AZD6244, breastfeeding should be discontinued if the mother is treated with AZD6244. Participants with unstable or serious medical conditions such as uncontrolled diabetes, uncontrolled hypertension, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, uncontrolled cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements are excluded due to the possibility that the underlying condition may obscure the attribution of effect and adverse events and may limit study compliance. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with AZD6244. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

7.3 Participation of Children

This study includes patients 18 years of age and older. Because insufficient dosing or adverse event data are currently available on the use of AZD6244 in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials. Studies will be performed in patients <18 years of age when it is appropriate to do so.

7.4 Evaluation of Benefits and Risks/Discomforts

There may or may not be any clinical benefit to a patient from participation in this trial. Their participation will benefit future cancer patients. Potential risks include the possible occurrence of any of a range of side effects that are listed in the consent document. It is possible that the side effects of radiation treatment and Capecitabine may be increased with AZD6244. The procedure for protecting against or minimizing risks will be to exclude patients with known contraindications to this therapy and to medically evaluate patients as described in protocol Section 3.3. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which participants are entitled under applicable regulations.

Optional biopsies will be obtained during sigmoidoscopy. The risks of sigmoidoscopy include discomfort during the procedure, bleeding, infection, gas, and the remote risk of perforation.

7.5 Consent and Assent Process and Documentation

An associate or principal investigator on the trial will inform patients of the purpose, alternatives, drug administration plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient. The original signed consent goes to Medical Records; a copy will be placed in the research record. All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

7.6 Procedure for Protecting Against or Minimizing Any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will be monitored for side effects from taking study medication. This research represents a greater than minimal risk to participants, but presents the prospect of direct benefit to individual subjects.

7.6.1 Patient Advocate

The patients' rights representative is available to patients receiving treatment on this protocol at the NIH Clinical Center at (301) 496-2626 in Building 10 of the Clinical Research Center, Room 1-3521, on the Bethesda NIH campus. Patients will be informed that they can contact the study PI or RN at any time with questions about their medical care, and that the patients' rights representative is also available to answer nonmedical questions about the study.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 6.1.

8.1 CTEP-Supplied Investigational Agent(s)

8.1.1 AZD6244 Hydrogen Sulfate (NSC 748727)

- **Chemical Name:** 6-(4-Bromo-2-chloro-phenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxy-ethoxy)-amide hydrogen sulfate
- **Other Names:** ARRY-142886; AR00142866; AR-142886-01
- **Classification:** Mitogen-activated protein kinase (MEK) inhibitor
- **CAS Registry Number:** 943332-08-9
- **Molecular Formula:** $C_{17}H_{15}BrClFN_4O_3 \cdot H_2SO_4$
- **M.W.:** 555.7
- **Solubility:** Solubility in Captisol® approximately 1 mg/mL at 25°C. Very low aqueous solubility (3.4 mcg/mL at pH 7.4).
- **Mode of Action:** The RAS/RAF/MEK/ERK pathway is an important mediator of many cellular processes including proliferation, survival, differentiation, apoptosis, motility, and metabolism. This pathway is often aberrantly activated in human tumors due to the overexpression of activated K-RAS, mutant b-Raf, or other growth factor receptors. AZD6244 is a selective mitogen-activated protein kinase (MEK) inhibitor. By inhibiting MEK, AZD6244 inhibits ERK phosphorylation. Thus, AZD6244 may inhibit oncogenic growth signaling in tumor cells by targeting the RAS/RAF/MEK/ERK pathway.
- **How Supplied:** AZD6244 hydrogen sulfate is supplied as a 25 mg, size 4, plain, white, hydroxypropylmethylcellulose (HPMC) capsule in white high density polyethylene (HDPE) containers with foil-lined, induction-sealed, child-resistant closures. Each bottle contains 60 capsules. Each capsule contains a dispersion of AZD6244 hydrogen sulfate in d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS; a water soluble form of vitamin E).
- **Storage:** Store the AZD6244 hydrogen sulfate capsules at room temperature (20°C-25°C). Brief excursions are permitted between 15°C and 30°C.
- **Stability:** Stability studies are ongoing.
- **Route of Administration:** Oral. Take AZD6244 capsules on an empty stomach

(either 1 hour before or 2 hours after meals). AZD6244 capsules should be taken with water only.

- **Potential Drug Interactions:** AZD6244 is primarily metabolized by CYP1A2 to N-desmethyl AZD6244 which is 3-5 fold more pharmacologically active than AZD6244.

  In vitro, AZD6244 is metabolized to a lesser extent by CYP2C19 and CYP3A4.
  In vitro, AZD6244 is a weak inhibitor of CYP2C9 and CYP1A2.

High vitamin E doses may potentiate warfarin's anticoagulant activity. Monitor PT/INR more frequently in patients receiving both warfarin and AZD6244 hydrogen sulfate capsules. Avoid concomitant intake of Vitamin E in excess of 100% of the recommended daily dose.

8.1.2 Agent Ordering

AZD6244 (NSC 748727) may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Agent may be requested by completing a Clinical Drug Request (NIH986) and faxing it to the Pharmaceutical Management Branch at (301) 480-4612. For questions about drug orders, transfers, returns, or accountability call (301) 496-5725 Monday through Friday between *:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.3 Agent Accountability

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the CTEP home page at http://ctep.cancer.gov for the Procedures for Drug Accountability and Storage and to obtain a copy of the DARF and Clinical Drug Request form.)

8.2 **Commercial Agent(s):**

8.2.1 Capecitabine

Chemical Name: 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl]-cytidine
Other Names: xeloda
Classification: Fluoropyrimidine carbamate
M.W.: 359.35

Solubility: Aqueous solubility of 26 mg/mL at 20ºC.
Mode of Action: Capecitabine is readily absorbed in the gastrointestinal tract and hydrolyzed in the liver to 5'-deoxy-5-fluorocytadine (5'-DFCR). Cytidine deaminase converts 5'-DFCR to 5'-deoxy-5-fluorodine (5'-DFUR) which is further hydrolyzed to the active drug 5-FU. 5-FU is metabolized to 5-fluoro-2'deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FdUMP binds covalently to thymidylate synthase and thereby inhibits the formation of thymidylate from 2'-deoxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, so that a deficiency of this compound can inhibit cell division. Nuclear enzymes can mistakenly incorporate FUTP in place of uridine triphosphate during synthesis of RNA which can interfere with RNA processing and protein synthesis.
How Supplied: Capecitabine is supplied as a biconvex oblong film coated caplet containing either 150 mg of capecitabine or 500 mg of capecitabine. The inactive ingredients in capecitabine include anhydrous lactose, croscarmellose sodium, hydroxypropyl methycellulose, microcrystalline cellulose, magnesium stearate, and purified water. The film coating contains hydroxypropyl cellulose, talc, titanium dioxide, and synthetic yellow and red iron oxides.
Storage: Store at 25° C; excursions permitted to 15 to 30° C.
Route of Administration: Oral.
Potential Drug Interactions: Administration with Warfarin can increase the INR beyond expected. Capecitabine should not be administered with aluminum hydroxide or magnesium hydroxide containing antacids, such as Maalox, as it may increase the plasma concentrations of capecitabine and its metabolites.
Source: Capecitabine will be purchased from commercial sources by the NIH Clinical Center Pharmacy Department.
Note: The reader is referred to the capecitabine package insert for additional information.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Tumor Biopsies

9.1.1.1 Tumor mutational status – *Laboratory Correlative Study #1*

- Collection of specimens: Biopsy specimen will be obtained at the time of pretreatment endoscopy. If sufficient tissue is available for this study from the initial diagnostic biopsy, this biopsy will not be performed.
- Handling of Specimens: biopsies will be collected, immediately placed in sterile saline on wet ice, and transported to the laboratory of Deborah Citrin. Specimens will centrifuged to remove supernatant and frozen at -80 C until use.

9.1.1.2 Tumor ERK1/2 phosphorylation – *Laboratory Correlative Study #2*)

- Collection of Specimen: Specimen will be collected at the time of pretreatment endoscopy and on day 8 and day 15. If sufficient tissue is available for this study from the initial diagnostic biopsy, the pretreatment biopsy will not be performed

- Handling of Specimens: The specimen will be submitted to pathology in formalin for processing and immunohistochemistry.

9.1.2 Research Labs

9.1.2.1 Peripheral mononuclear cell ERK1/2 phosphorylation – *Laboratory Correlative Study #3*)

- Collection of Specimen: A whole blood specimen will be collected pretreatment, day 8, day 15, and at four weeks of follow up (week 11). Nurses at the Radiation Oncology clinic will collect whole blood in one 10 cc EDTA tube.
- Handling of Specimens: Whole blood will be collected and transported immediately to the laboratory of Deborah Citrin for processing for flow cytometric analysis.

9.1.2.2 Plasma TGFα – *Laboratory Correlative Study #4*)

- Collection of Specimen: Specimen will be collected pretreatment, day 8, day 15, and at four weeks of follow up (week 11). Nurses at the Radiation Oncology clinic will collect whole blood in one 10 cc EDTA tube for plasma.
- Handling of Specimens: Blood for plasma will be immediately transported on ice to the laboratory of Deborah Citrin. Plasma will be processed within 15 minutes of collection. Aliquots will be stored at -80 C until use.

9.2 Sample processing

Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality pursuant to informed consent provisions.

Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. To ensure patient confidentiality, only containers used for the initial specimen collections will be labeled with patient identifiers. Only the barcode identifier will be applied to all subsequent specimen containers. When specimens are processed and aliquoted, no patient information will be included on the new containers. Original specimen containers will be discarded. Only barcode-labeled specimens without patient identifiers will be sent for analysis and/or storage. Specimen labels will indicate: protocol number, order in which the patient enrolled on the trial, type of sample, collection time, and total volume collected, as appropriate. Samples from sets of at least three patients will be grouped for scientific analysis.

The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. The only patient information available in the inventory system will be the patient sex, diagnosis, and level of informed consent given. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory

system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e., broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Any new use of these samples will require prospective IRB review and approval; any loss or destruction of samples and the planned disposition of samples after the protocol is terminated will be reported to the IRB.
All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss.

If the patient withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk[h] 11	Follow up 3, 6, 9, 12, 24, and 36 months after completion of chemoradiation
AZD6244		A	A	A	A	A	A	A				

Capecitabine			B	B	B	B	B	B				
Radiation			C	C	C	C	C	C				
Informed consent	X											
Ophthalmologic examination[e]	X[f]											
Surgical Consult	X[f]											
Demographics	X											
Medical history	X											
Concurrent meds	X	X	X	X	X	X	X	X			X	X
Physical exam	X	X	X	X	X	X	X	X			X	X
Vital signs with pulse oximetry	X	X	X	X	X	X	X	X			X	
Height	X											
Weight	X										X	X
Performance status	X											
CBC w/diff, plts, PT/PTT	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[a]	X	X	X	X	X	X	X	X	X	X	X	X
EKG	X	X[i]	X[i]		X[i]							
Adverse event evaluation	X	X	X	X	X	X	X	X	X	X	X	X[g]
Tumor measurements	X										X	
Radiologic evaluation	X										X	X
Pathology	X											
CT (CAP)	X										X	X
CEA	X											
Endoscopy	X											
B-HCG	X[b]											
Optional tumor biopsy	X[d]		X	X								
PKs		X	X									
Research blood draw	X		X	X							X	

A: AZD6244: Dose as assigned; po BID dailyB: Capecitabine, Dose 825 mg/m2 po every 12 hours, Monday- Friday (2 hours prior to radiation and 12 hours after first dose)
C Radiotherapy: daily Monday- Friday

a Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Serum pregnancy test (women of childbearing potential).
c: Off-study evaluation.
d: If sufficient tissue is available for pretreatment correlative work from the initial diagnostic biopsy this procedure will not be performed.
e. Opthalmologic evaluation will be repeated in the event of any visual change or visual event due to AZD6244.
f. Baseline ophthalmologic and surgical consultation should be obtained within 3 weeks of beginning protocol therapy.
g. RTOG scoring only
h. Week 11 is pre- surgery
i. EKG will be obtained on Day 1 (2 hours after first AZD6244 dose), Day 8, week 4 and in the case of a cardiorespiratory AE.

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated

at week 11 just prior to surgical therapy. After surgical therapy, only recurrence will be documented. Surgical and pathologic findings will be used to confirm response assessment at 11 weeks.

11.1 **Antitumor Effect – Solid Tumors**

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (version 1.1) [Eur J Cancer. 2009 Jan;45(2):228-47].Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

11.1.1 Definition

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with AZD6244.

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

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (x-ray) or as ≥10 mm with spiral CT scan or caliper measurements by clinical exam. Lymph nodes must be ≥15 mm in short axis by CT to be considered measurable (for lymph nodes only short axis will be followed). All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Target lesions. All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions. All other lesions (or sites of disease) including any measurable

lesions over and above the 10 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

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

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

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

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

Conventional CT and MRI These techniques should be performed with cuts of 5 mm or less in slice thickness contiguously. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols. MRI is acceptable in certain situations.

Ultrasound (US) When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical

trials are being developed.

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

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

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

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

11.1.4.2 Evaluation of Non-Target Lesions

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

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

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

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

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

Principal Investigator).

11.1.4.3 Evaluation of Best Overall Response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

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

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

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

11.1.5 Duration of Response

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

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

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

11.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression.

11.2 **Other Response Parameters**

Pathologic complete response will be assessed on surgical specimens. This will include an evaluation of evidence of residual gross and microscopic disease in the resected rectum and

lymph nodes.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. Instructions for submitting data using the CDUS can be found on the CTEP web site (http://ctep.cancer.gov/reporting/cdus.html).

Note: **All** adverse events that have occurred on the study, including those reported through AdEERS, must be reported via the monitoring method identified above.

12.1.2 Responsibility for Data Submission

N/A

12.2 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be

referred to as "Multi-Party Data."):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used, and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least

three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
Executive Plaza North, Suite 7111
Bethesda, Maryland 20892
FAX 301-402-1584
Email: anshers@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 **Study Design/Endpoints**

This is a Phase I trial in which patients will be treated with escalating doses of AZD6244. There will be no intrapatient dose escalation. The primary endpoints will be the MTD and the DLT's. The dose-escalation will proceed in cohorts of 3-6 patients at each dose level. If 0/3 experience DLT, the next cohort starts one dose level higher. If 1/3 experience DLT, up to 3 more patients are started at that same dose level. If 2 or more experience DLT, no further patients are started at that dose. In the event that 2 or more patients of the first three enrolled at dose level 1 develop a DLT, de-escalation to dose level -1 will occur. The MTD or recommended Phase II dose is the dose level at which no more than 1 of 6 patients experience DLT during treatment and the three weeks after completion. Six additional patients will be treated at the recommended phase II dose in order to refine toxicity assessment and obtain reasonable amounts of biologic data.

The dose escalation will proceed only for AZD6244 while capecitabine and radiation doses remain constant. The schema for escalation is as follows:

Dose Escalation Schedule			
Dose Level	**Dose**		
	AZD6244	***Capecitabine***	***Radiation****
Level -1	50 mg PO QD Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
Level 1	50 mg PO BID Weeks 1-7	825 mg/m^2 PO every 12 hours Weeks 2-7	5040 cGy Weeks 2-7
Level 2	75 mg PO BID Weeks 1-7	825 mg/m^2 PO every 12 hours	5040 cGy Weeks 2-7

		Weeks 2-7	
* An additional 360 cGy may be delivered for fixed T4 tumors for a total dose of 5400 cGy			

Toxicity will be scored according to the CTCAE v4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev4.pdf). Dose-limiting toxicities will be defined as follows (during treatment and within the first three weeks after treatment)

1. Recurring and persistent Grade 3 diarrhea despite appropriate medical therapy.
2. ANC <500 for more than 5 days or neutropenic fever
3. Grade 4 thrombocytopenia
4. Recurring and persistent Grade 3 nausea/vomiting despite appropriate anti-emetic therapy
5. Grade 4 dermatitis acneiform rash
6. Grade 4 fatigue
7. All other non-hematologic toxicities of grade 3 or higher except electrolyte abnormalities that are correctable
8. Delays in radiation treatment due to toxicity of more than 2 weeks (cumulative duration over the course of the entire treatment).

Toxicities should be attributable (possible, probable or definite) to the AZD6244, Capecitabine, and RT to constitute DLT. Patients who develop a DLT will continue Capecitabine and radiation after resolution or partial resolution of the DLT as clinically appropriate, and dose reduction of AZD6244 will occur as in section 5.1.

13.2 Sample Size/Accrual Rate

The planned sample size is a minimum of 4 and a maximum of 18. The estimated accrual is two patients per month.

13.3 Analysis of Secondary Endpoints

The secondary endpoints include pathologic CR at the time of surgical resection, pERK/total ERK in PBMC and tumor, plasma TGFα level, pharmacokinetics of AZD6244 alone compared to in combination with capecitabine, and presence of mutations in tumor biopsies. Analysis of secondary endpoints will focus on samples obtained at the MTD (12 patients) with the goal of describing potentially useful measures for future testing in Phase II trials of sufficient power. Changes in TGFα level from pre to post treatment will be related to the probability of pathologic CR using logistic regression. Presence of activating *BRAF* or *RAS* mutations or inactivating PTEN mutations in tumor biopsies pretreatment will be related to the probability of pathologic CR using logistic regression. We will evaluate if a reduction in pERK in PBMC (flow cytometry) correlates to a reduction in pERK in tumor (IHC grading) after AZD6244 treatment with a Spearman Rank Correlation.

The final PK analyses will be the responsibility of Clinical Pharmacology and DMPK, Alderley Park, AstraZeneca, UK. The AZD6244, N-desmethyl and AZD6244 amide concentration-time data, along with the derived PK parameters, will be listed and summarized appropriately. Where data allow, the following PK parameters will be

determined following administration of AZD6244 alone and following administration of AZD6244 alone and AZD6244 & capecitabine together. Additional parameters may be determined if deemed appropriate:

AZD6244: C_{max}, t_{max}, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $C_{min,}$ CL/F

N-desmethyl AZD6244 and AZD6244 amide: C_{max}, t_{max}, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t),}$ C_{min}, CL/F, AUC metabolite:parent ratio, C_{max} metabolite:parent ratio

The PK parameters will be derived using non-compartmental analysis. The maximum plasma concentrations (C_{max}) and the time to reach the maximum plasma concentrations (t_{max}) will be determined by visual inspection of the plasma concentration-time profiles. The area under the plasma concentration-time curve from zero to 12 hours post dose $AUC_{(0-12)}$ and zero to 24 hours post-dose $AUC_{(0-24)}$ will be calculated by the linear trapezoidal rule. The total oral clearance from plasma (CL/F) after an oral dose will be calculated from the dose divided by the AUC during the appropriate dosing interval.

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APPENDIX A

Performance Status Criteria

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

APPENDIX B

New York Heart Association Classifications

Clinical Evaluation of Functional Capacity of Patients
with Heart Disease in Relation to Ordinary Physical Activity

Class	Cardiac Symptoms	Limitations	Need for Additional Rest*	Physical Ability to work**
I	None	None	None	Full time
II	Only moderate	Slight	Usually only slight or occasional	Usually full time
III	Defined, with less than ordinary activity	Marked	Usually moderate	Usually part time
IV	May be present even at rest, and any activity increases discomfort	Extreme	Marked	Unable to work

* To control or relieve symptoms, as determined by the patient, rather than as advised by the physician.

** At accustomed occupation or usual tasks.

Reference: Bruce, R. A.: Mod. Concepts Cardiovasc. Dis. 25:321, 1956.
(Modified from New York Heart Association, 1953).

APPENDIX C

MEDICATIONS THAT MAY CAUSE QTc PROLONGATION

The following table presents a list of drugs that may prolong the QTc. These drugs are prohibited during the study. AZD 6244 may be administered after a 5 half-life washout period elapses following the use of these drugs. Washout period is based on roughly 5 half-lives and rounded to a convenient interval.

Compound	Compound Half Life	Possible Washout Period - Hours	Possible Washout Period - Days
Alfuzocin	~10 hours		7
Amantadine	17 +/- 4 hours (10-25)		4
Amiodarone (cordarone)	58 days (15-142) 36 days (active metabolite)		180
Amitriptyline*	> 24 hours, wide interpatient variability		
Arsenic trioxide	Not characterized		
Azithromycin	40 hours		
Bepridil	42 hr (26-64)		10
Chloral hydrate	Readily converted to Trichloroethanol (active metabolite $T_{1/2}$=7-10 hour)	48	
Chloroquine	Prolonged (days to weeks)		
Chlorpromazine	30 +/- 7 hours		7
Cisapride	6 – 12 hour, up to 20 hour	60	
Clarithromycin	Non linear PK3-4 hr (250mg Q12) 5-7 hr (500mg Q12)	36	
Cloroquine	6 to 60 days; mean 20 days		
Desipramine*	> 24 hours, wide interpatient variability		
Disopyramide	6.7 hr (4-10)	36	
Dofetilide	10 hr	48	
Dolesetron	8.1 hr		
Domperidone	7-8 hr	48	
Doxepin*	> 24 hours, wide interpatient variability		
Droperidol	2.2 hours	10	
Erythromycin	* Each salt form has different Half life*		
Felbamate	20-23 hr		5
Flecainide	20 hr (12-27)		5
Foscarnet	87.5+/-41.8 hours *distribution and release from bone*		20
Fosphenytoin	12-29 hr		6
Gatifloxacin	7-14 hr	48	
Gemifloxacin	7 hours	48	
Grepafloxacin	16 hr		3
Halofantrine	6-10 days (variable among individual)		45
Haloperidol	18 +/-5 hr		5
Ibutilide	6 hours (2-12) * variable among subject*	36	
Imipramine*	> 24 hours, wide interpatient variability		
Indapamide	14 hours (biphasic elimination)		3
Isradipine	8 hours (multiple metabolites)	48	
Levofloxacin	6-8 hours	48	
Levomethadyl	Multiple compartment PK with active metabolite 2.6 day for LAAM, 2 day for nor-LAAM, 4 day for dinor-LAAM		20
Lithium	24 hour (10-50)		7

Compound	Compound Half Life	Possible Washout Period - Hours	Possible Washout Period - Days
Mesoridazine	24-48 hours (animal study)		10
Methadone	15-30 hours		7
Moexipril/HCTZ	2-9 hour (include active metabolite) for moexipril; 5.6-14.8 hours for HCTZ	48	
Moxifloxacin	12 +/-1.3 hours	72	
Naratriptan	6 hours	36	
Nicardipine	~ 2 hour post IV infusion	12	
Nortriptyline*	> 24 hours, wide interpatient variability		
Octreotide	1.7 hours	12	
Ofloxacin	5 to 7.5 hours		2
Ondansetron	4 hours (IV/IM); 3 hours (PO)		1 to 3
Pentamidine	6.4+/-1.3 hours	36	
Pimozide	55 hours		10
Procainamide	3-4 hour for PA and NAPA (active metabolite)	24	
Protiptyline*	> 24 hours, wide interpatient variability		
Quetiapine	6 hours	36	
Quinidine	6-8 hours in adult; 3-4 hours in children	36	
Quinine	4-5 hours		
Risperidone	3-20 hours (extensive to poor metabolizer) 9-hydroxyrisperidone (active metabolite) T ½ =21-30 hours (extensive to poor metabolizer)		4
Salmeterol	5.5 hours (only one datum)	36	
Sotalol	12 hours	72	
Sparfloxacin	20 hours (16-30)		4
Sumatriptan	2.5 hours	12	
Tacrolimus	~34 hours in healthy; ~19 hours in Kidney transplant		7
Tamoxifen	5-7 days (biphasic)		30
Telithromycin	2-3 hr	24	
Thioridazine	20-40 hours (Phenothiazines)		7
Tizanidine	2.5 hours	12	
Vardenifil	4 to 5 hours		
Venlaflaxine	5 +/-2 hours for parent comp. 11+-2 hours for OVD (active metabolite)	60	
Voriconizole	6 hours; dose dependent		
Ziprasidone	7 hr	36	
Zolmitriptan	2.8-3.7 hours (higher in female)	18	

***** Weakly associated with Torsades de Pointes and/or QT prolongation but that are unlikely to be a risk for Torsades de Pointes when used in usual recommended dosages and in patients without other risk factors (e.g., concomitant QT prolonging drugs, bradycardia, electrolyte disturbances, congenital long QT syndrome, concomitant drugs that inhibit metabolism).

References:

1. **Physician's Desk Reference 2002**
2. **Facts and Comparisons (update to June 2005)**
3. **The Pharmacological Basis of Therapeutics 9th Edition, 1996**

APPENDIX D: Patient's Medication Diary

Patient name_____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form for both AZD6244 and Capecitabine.
2. You must take AZD6244 on an empty stomach with water only, either 1 hour before or two hour after meals. Capecitabine should be taken two hours before radiation with food.
3. Record the date, the number of pills you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
3. Please bring this form to your physician when you go for your weekly appointment.
4. Total dose of AZD6244: ___mg ____ times per day. Total number of pills at each dose: ___.
5. Total dose of capecitabine:_____mg two times per day. ____ 150 mg pills and ____500 mg pills at each dose.

		AZD6244		Capecitabine 150 mg		Capecitabine 500 mg		Comments
Date	Day	# pills, when taken	# of pills missed	# pills, when taken	# of pills missed	# pills, when taken	# of pills missed	
	1			Capecitabine is not taken on these days				
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13			Capecitabine is not taken on these days				
	14							
	15							
	16							
	17							
	18							
	19							
	20			Capecitabine is not taken on these days				
	21							

Patient's signature_____ Date_____

Patient name_____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form for both AZD6244 and Capecitabine.
2. You must take AZD6244 on an empty stomach with water only, either 1 hour before or two hour after meals. Capecitabine should be taken two hours before radiation and with food.
3. Record the date, the number of pills you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
3. Please bring this form to your physician when you go for your weekly appointment.
4. Total dose of AZD6244: ___mg ____ times per day. Number of pills at each dose: ___.
5. Total dose of capecitabine:_____mg two times per day. ____ 150 mg pills and ____500 mg pills at each dose.

		AZD6244		Capecitabine 150 mg		Capecitabine 500 mg		Comments
Date	Day	# pills, when taken	# of pills missed	# pills, when taken	# of pills missed	# pills, when taken	# of pills missed	
	22							
	23							
	24							
	25							
	26							
	27			Capecitabine is not taken on these days				
	28							
	29							
	30							
	31							
	32							
	33							
	34			Capecitabine is not taken on these days				
	35							
	36							
	37							
	38							
	39							
	40							
	41			Capecitabine is not taken on these days				
	42							
	43							
	44							
	45							
	46							
	47							

Patient's signature_____ Date_____

APPENDIX E: Laboratory Correlative Studies Background

Tumor mutational status – *Laboratory Correlative Study #1*
The biopsy specimen will be obtained at the time of pretreatment endoscopy. Biopsies will be collected, immediately placed in sterile saline on wet ice, and transported to the laboratory of Deborah Citrin. Specimens will centrifuged to remove supernatant and frozen at -80 C until transfer to the Laboratory of Pathology. Evaluation for mutations will be performed by the Laboratory of Pathology at the NCI which completes these assays as standard practice on tumor samples. If additional DNA or tissue remains and PTEN expression is absent by immunohistochemistry, pcr for amplification of PTEN exons followed by sequencing will occur in the laboratory of Dr. Deborah Citrin.

Tumor ERK1/2 phosphorylation and PTEN expression – *Laboratory Correlative Study #2*)
The specimen will be collected at the time of pretreatment endoscopy and on day 8 and day 15. The specimen will be submitted in formalin for processing and imunohistochemistry. Immunohistochemistry will be performed with chromagen based detection using a polyclonal primary antibody directed against phospho-ERK1/2.(45, 46) If additional sample is available tumor PTEN expression will be evaluated with immunohistochemistry.

Peripheral mononuclear cell ERK1/2 phosphorylation and pharmacokinteics – *Laboratory Correlative Study #3*)
Collection of Specimen: A whole blood specimen will be collected pretreatment, day 8, day 15, and at one month of follow up. Whole blood will be collected and transported immediately to the laboratory of Deborah Citrin for processing for flow cytometric analysis. The technique used for processing and analysis will be identical to that used by Astra Zeneca in prior trials of AZD6244.

PBMC and plasma samples will also be collected on days 4-5 and days 11-12 for pharmacokinetic and pharmacodynamic analysis. Collection of specimen: 3 mL of whole blood will be sampled at the time points indicated below. Blood will be immediately transported to the laboratory of Deborah Citrin for processing and storage prior to shipment in batches to Astra Zeneca.

For QD dosing these samples will be obtained at): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8, 12 and 24 hours post-dose (24 hour sample is pre- the next day's dose). Day 11 or 12 (AZD6244 + capecitabine + radiotherapy): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8, 12 and 24 hours post-dose (24 hour sample is pre- the next day's dose).

For BID dosing these samples will be obtained at Day 4 or 5 (AZD6244 alone): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8 and 12 hours post-dose (12 hour sample is pre- the next day's dose). Day 11 or 12 (AZD6244 + capecitabine + radiotherapy): Pre-dose (within 10 minutes of dosing), 30 minutes, 1 hour, 1 hour 30 minutes, 2, 4, 8 and 12 hours post-dose (12 hour sample is pre- the next day's dose).

Plasma TGFα – *Laboratory Correlative Study #4*)Collection of Specimen: Specimen will be collected pretreatment, day 8, day 15, and at one month of follow up. Plasma will be immediately transported on ice to the laboratory of Deborah Citrin. Plasma will be processed within 15 minutes of collection. Aliquots will be stored at -80 C until use. TGF-α levels will be assessed with the commercially available Elisa kit from R&D Systems as per manufacturer's instruction

APPENDIX F: ACUTE RTOG

	[0]	[1]	[2]	[3]	[4]
SKIN	No change over baseline	Follicular, faint or dull erythema/ epilation/dry desquamation/ decreased sweating	Tender or bright erythema, patchy moist desquamation/ moderate edema	Confluent, moist desquamatiom other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis
UPPER G.I.	No change	Anorexia with <=5% weight loss from pretreatment baseline/ nausea not requiring antiemetics/ abdominal discomfort not requiring parasympatholytic drugs or analgesics	Anorexia with <=15% weight loss from pretreatment baseline/nausea &/ or vomiting requiring antiemetics/ abdominal pain requiring analgesics	Anorexia with >15% weight loss from pretreatment baseline or requiring N-G tube or parenteral support. Nausea &/or vomiting requiring tube or parenteral support/abdominal pain, severe despite medication/hematemesis or melena/ abdominal distention (flat plate radiograph demonstrates distended bowel loops	Ileus, subacute or acute obstruction, performation, GI bleeding requiring transfusion/abdominal pain requiring tube decompression or bowel diversion
LOWER G.I. INCLUDING PELVIS	No change	Increased frequency or change in quality of bowel habits not requiring medication/ rectal discomfort not requiring analgesics	Diarrhea requiring parasympatholytic drugs (e.g., Lomotil)/ mucous discharge not necessitating sanitary pads/ rectal or abdominal pain requiring analgesics	Diarrhea requiring parenteral support/ severe mucous or blood discharge necessitating sanitary pags/abdominal distention (flat plate radiograph demonstrates distended bowel loops)	Acute or subacute obstruction, fistula or perforation; GI bleeding requiring transfusion; abdominal pain or tenesmus requiring tube decompression or bowel diversion
GENITOURINARY	No change	Frequency of urination or nocturia twice pretreatment habit/ dysuria, urgency not requiring medication	Frequency of urination or nocturia which is less frequent than every hour. Dysuria, urgency, bladder spasm requiring local anesthetic (e.g., Pyridium)	Frequency with urgency and nocturia hourly or more frequently/ dysuria, pelvis pain or bladder spasm requiring regular, frequent narcotic/gross hematuria with/ without clot passage	Hematuria requiring transfusion/ acute bladder obstruction not secondary to clot passage, ulceration or necrosis
HEMATOLOGIC WBC (X 1000)	>=4.0	3.0 - <4.0	2.0 - <3.0	1.0 - <2.0	<1.0
PLATELETS (X 1000)	>=100	75 - <100	50 - <75	25 - <50	<25 or spontaneous bleeding
NEUTROPHILS	>=1.9	1.5 - <1.9	1.0 - <1.5	0.5 - <1.0	<0.5 or sepsis
HEMOGLOBIN (GM %)	>11	11-9.5	<9.5 - 7.5	<7.5 - 5.0	-------
HEMATOCRIT (%)	>=32	28 - <32	<28	Packed cell transfusion required	-------

GUIDELINES: The acute morbidity criteria are used to score/grade toxicity from radiation therapy. The criteria are relevant from day 1, the commencement of therapy, through day 90. Thereafter, the EORTC/RTOG Criteria of Late Effects are to be utilized.

The evaluator must attempt to discriminate between disease- and treatment-related signs and symptoms.

An accurate baseline evaluation prior to commencement of therapy is necessary.

All toxicities Grade 3, 4 or 5* must be verified by the Principal Investigator.

*ANY TOXICITY WHICH CAUSED DEATH IS GRADED 5.

APPENDIX G: LATE RTOG TOXICITY

	[0]	[1]	[2]	[3]	[4]
SKIN	No change	Slight atrophy Pigmentation change Some hair loss	Patch atrophy; Moderate telangiectasia; Total hair loss	Marked atrophy; Gross telangiectasia	Ulceration
SUBCUTANEOUS TISSUE	No change	Slight induration (fibrosis) and loss of subcutaneous fat	Moderate fibrosis but asymptomatic Slight field contracture <10% linear reduction	Severe induration and loss of subcutaneous tissue Field contracture >10% linear measurement	Necrosis
SMALL/LARGE INTESTINE	No change	Mild diarrhea Mild cramping Bowel movement 5 times daily Slight rectal discharge or bleeding	Moderate diarrhea and colic Bowel movement >5 times daily Excessive rectal mucus or intermittent bleeding	Obstruction or bleeding requiring surgery	Necrosis/ Perforation Fistula
BLADDER	No change	Slight epithelial atrophy Minor telangiectasia (microscopic hematuria)	Moderate frequency Generalized telangiectasia Intermittent macroscopic hematuria	Severe frequency and dysuria Severe generalized telangiectasia (often with petechiae) Frequent hematuria Reduction in bladder capacity (<150 cc)	Necrosis/ Contracted bladder (capacity <100 cc) Severe hemorrhagic cystitis
BONE	No change	Asymptomatic No growth retardation Reduced bone density	Moderate pain or tenderness Growth retardation Irregular bone sclerosis	Severe pain or tenderness Complete arrest of bone growth Dense bone sclerosis	Necrosis/ Spontaneous fracture
JOINT	No change	Mild joint stiffness Slight limitation of movement	Moderate stiffness Intermittent or moderate joint pain Moderate limitation of movement	Severe joint stiffness Pain with severe limitation of movement	Necrosis/ Complete fixation

GUIDELINES: The late morbidity criteria are used to score/grade toxicity from radiation therapy. The critera are relevant beginning at day 90 after therapy.

The evaluator must attempt to discriminate between disease- and treatment-related signs and symptoms.

An accurate baseline evaluation prior to commencement of therapy is necessary.

All toxicities Grade 3, 4 or 5* must be verified by the Principal Investigator.

*ANY TOXICITY WHICH CAUSED DEATH IS GRADED 5.