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TITLE: A Phase II Study of Lapatinib for the Treatment of Stage IV Melanoma harboring *ERBB4* Mutations

Short Title: Lapatinib for melanoma

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PRECIS

Background:

- Patients with stage IV melanoma have few available treatment options and an overall poor prognosis.
- Pre-clinical evidence suggests that lapatinib has activity against metastatic melanoma harboring *ERBB4* mutations.

Objectives:

Primary Objectives:

- Determine the response rate to lapatinib administered as 500 mg orally twice daily on a continuous schedule in patients with metastatic melanoma harboring *ERBB4* mutations.

Secondary Objectives:

- To determine the progression free survival of patients with stage IV melanoma treated with lapatinib monotherapy.
- To evaluate the safety of lapatinib in patients with metastatic melanoma
- To determine the impact of additional genetic alterations on the response to lapatinib in melanoma harboring *ERBB4* mutations
- To develop a clinically applicable biomarker predictive of response to lapatinib in patients with melanoma harboring *ERBB4* mutations
- To determine the pharmacokinetics of lapatinib administered as 500 mg orally twice daily on a continuous schedule in patients with metastatic melanoma harboring *ERBB4* mutations

Eligibility:

- Patients ≥ 18 years of age with stage IV melanoma, who have measurable disease and whose tumors express up to two *ERBB4* gene mutations.
- Patient must be ECOG performance status of ≤ 1 and a life expectancy of more than 3 months.
- Patients must have adequate organ function.
- Patients must not have had surgery, chemotherapy, hormonal therapy, radiation therapy, or biological therapy for at least 4 weeks prior to starting study medication.
- Patients must not have an acute, critical illness.
- All patients who are sexually active and able to conceive will be required to use contraception during treatment with lapatinib.

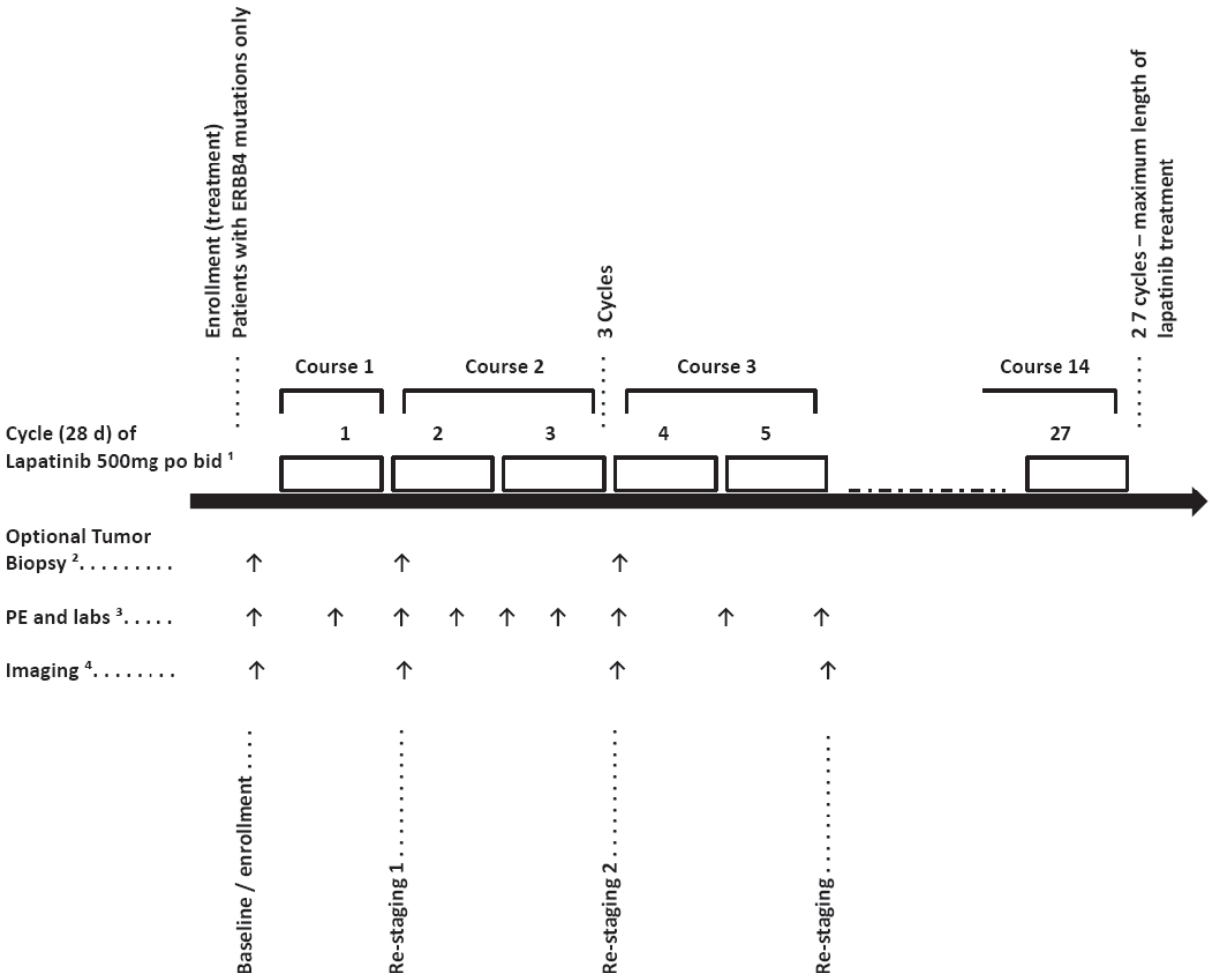
Design:

- Patients will be screened for the presence of *ERBB4* gene mutations in their tumor and only patients who harbor ≤2 *ERBB4* mutations will be enrolled in the treatment phase of the study.
- Lapatinib will be administered as an oral dose of 500 mg twice daily (in the morning and evening) taken one hour before or after meals. Lapatinib will be given continuously; one cycle equals 28 days. Course 1 equals cycle 1; all subsequent courses are 8 weeks long

(2 cycles). A patient may receive up to 27 cycles (14 courses).

- Up to 25 patients (allowing for a staged accrual of initially 16 patients who will receive lapatinib and are evaluable after the 1st cycle) will be enrolled over 2-3 years and the trial will be completed over 3-5 years, allowing for completion of follow-up.
- The primary objective of the trial will be to determine whether lapatinib monotherapy in this setting is able to be associated with a response rate (PR +CR) that can rule out 10% (p0=0.10) in favor of an improved response rate of 30% (p1=0.30).

TREATMENT SCHEMA



[1] Lapatinib (GW2016) will be self-administered orally (po) 500mg bid; for 28 days in continuous cycles until disease progression occurs or the patient meets off-study criteria for a maximum of two years.
[2] Optional tumor biopsies will be collected pretreatment and on day 14 of cycle 1 (+/- 5 days) and on day 28 of cycle 3 (+/- 5 days).
[3] Physical examination (PE) and laboratory blood investigations will be performed bi-weekly for the first 3 cycles and then monthly thereafter
[4] CT, MRI, or ultrasound imaging will be performed pretreatment, on day 28 of cycle 1 (+/- 5 days), on day 28 of cycle 3 (+/- 5 days), and then after every other two completed cycles thereafter

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1. OBJECTIVES

1.1. Primary Objectives

- Determine the response rate to lapatinib administered as 500 mg orally twice daily on a continuous schedule in patients with metastatic melanoma harboring *ERBB4* mutations.

1.2. Secondary Objectives

- To determine the progression free survival of patients with stage IV melanoma treated with lapatinib monotherapy.
- To evaluate the safety of lapatinib in patients with metastatic melanoma
- To determine the impact of additional genetic alterations on the response to lapatinib in melanoma harboring *ERBB4* mutations
- To develop a clinically applicable biomarker predictive of response to lapatinib in melanoma harboring *ERBB4* mutations
- To determine the pharmacokinetics of lapatinib administered as 500 mg orally twice daily on a continuous schedule in patients with metastatic melanoma harboring *ERBB4* mutations

2. BACKGROUND

2.1 Melanoma

Melanoma skin cancer is currently the most rapidly rising solid organ cancer in both men and women in the Caucasian U.S population.[1, 2] Predicted lifetime risk in the U.S. has risen to 1:58 in Caucasian males and 1:25 for Caucasian men in Australia.[1, 3] Overall, it is the sixth leading cancer in men and seventh most common in women with an annual incidence of 62,480 new cases and 8,420 deaths. Metastatic melanoma ranks second in terms of loss of years of potential life.[2] Metastatic melanoma has a poor prognosis with five-year survival of less than 5% with a median survival time of 6 months.[1, 2] The last time a novel treatment received regulatory approval for metastatic melanoma dates back to 1998:[4] FDA-approved treatments for metastatic melanoma include aldesleukin (IL-2) and dacarbazine (DTIC) chemotherapy.[5] Aldesleukin has an objective clinical response rate of about 16%, a complete response rate of 6%, and cures about 4% patients with metastatic melanoma.[6] Dacabarzine based chemotherapy has a clinical response rate of up to 20% but almost no complete responders and all patients eventually recur.[7, 8] Aside from dacarbazine, the only other single-agent chemotherapy shown to have impact on metastatic melanoma is temozolomide (TMZ), which is currently often given by many oncologists in the U.S. for off-protocol therapy for metastatic melanoma.[8]

Given the encouraging results of an immunologic approach to the treatment of metastatic melanoma with aldesleukin, several other immunotherapy-based

approaches are currently under investigation: anti-CTLA-4 blockade using either ipilimumab or tremelimumab yields response rates even lower than the ones achieved by conventional cytotoxic chemotherapy listed above, and their toxicity, in particular with respect to severe, potentially life-threatening autoimmune events does substantially limit their use.[9, 10] Adoptive cell therapy (ACT), as pioneered by the Surgery Branch/NCI, refers to an immunotherapy approach in which anti-tumor lymphocytes are identified and grown *ex vivo* and then infused back into the patient with or without preselection or genetic manipulation, often along with vaccines or growth factors that can augment the *in vivo* impact of the transferred cells.[11, 12] While with the use of preconditioning lymphodepletion this approach achieves impressive response rates of > 70% in select group of patients, only a limited number of patients are eligible and the tremendous financial and laboratory resources required prevents this approach currently from a more widespread application.[13]

Targeted therapy in melanoma

In view of the limited clinical efficacy of the treatment options listed above novel strategies on the development of targeted therapies that capitalize on the recent advances in the genetic understanding of melanoma pathogenesis have been pursued.[14] Many of the molecular therapy trials listed below, however, administered single agents in a non-selected manner negating the advantage of a genotype-directed treatment strategy which is likely why to date none of the novel targeted single agents evaluated in melanoma without additional patients' selection have shown any clinically meaningful activity.[15] This rather bleak outlook has only very recently started to change with novel molecular agents specifically targeting tumors harboring the BRAFV600 mutation or with the latest success of imatinib (Gleevec®) in melanoma harboring c-KIT mutations.[16, 17]

RTK/RAS/BRAF/MEK pathway

BRAF

The serine/threonine kinase BRAF of the MAPK pathway is mutated in approximately two-thirds of melanoma cases and constitutes the most commonly mutated oncogene in melanoma.[18, 19] BRAFV600 mutations have a 480-fold increase in the activity of BRAF over wild type, thus making it an attractive therapeutic target.[19]

The agent that has been most widely investigated in the context of targeted therapy against BRAF is the multikinase inhibitor sorafenib.[20, 21] In addition to BRAF, sorafenib targets Flt-3, CRAF, VEGF and PDGFβ. As a single agent, sorafenib has limited activity in melanoma, as demonstrated in a phase 2 trial showing no responses in 37 mostly heavily pretreated patients with metastatic melanoma.[22] Similar results have been obtained when sorafenib was combined with chemotherapy.[23]

MEK

The rationale for MEK inhibition as a prime target for melanoma stems from BRAF's downstream dependence on MEK for signal propagation. In a phase 1 study of 66

patients with various advanced malignancies including melanoma, C-1040 was found to be moderately effective.[24] A phase 1/2 trial of PD0325901, a second-generation MEK inhibitor enrolled 27 patients and demonstrated partial responses in 2/27 and stable disease in 5/27.[25] The compound AZD6244 is currently in phase II clinical trials where patients are selected upon presence of BRAFV600 mutation status.[26]

PTEN/PI3K/Akt3 pathway

The PI3K/Akt3 signaling pathway is aberrantly activated in up to 70 % of melanomas.[27] In preclinical models, strategies using single agent PI3K, Akt, mTOR, or combined PI3K/mTOR inhibition have shown to be successful.[28, 29] The approach to target direct substrates of Akt3, such as mTOR with temsirolimus and everolimus, is the clinically most advanced. However, in a phase 2 trial of 33 patients with metastatic melanoma, monotherapy with temsirolimus showed minimal efficacy. Similar results were seen in an interim analysis of a two-stage phase 2 trial using everolimus as monotherapy in patients with stage IV melanoma.[30]

c-KIT

Oncogenic mutations in c-KIT in melanoma follow a very heterogeneous pattern.[31] 39 % of melanomas at mucosal sites, 36 % at acral sites, and 28 % of chronically sun-damaged skin show oncogenic mutations or/and copy increases of KIT. The preferential involvement of c-KIT in these rarer types of melanoma, i.e. acral and mucosal, may explain the negative results of imatinib in an initial phase 2 trial of unselected patients.[32]

Despite published results of targeted therapy in melanoma being largely unsuccessful, two recent prime examples of successful genotype-directed therapy have recently started to change the outlook of molecular therapy in this disease: PLX4032 is a novel compound designed to target the BRAFV600 mutation in melanoma, where the TKI has substantially increased affinity to the mutated molecule compared to the wild type receptor.[33] PLX 4032 has more than a 1000-fold increased sensitivity to mutant BRAFV600 than wild type BRAF kinase, and initial results from several trials underway have shown objective response rates of greater than 70% in patients who harbor this mutation.[16, 33] Similarly, recent findings of a genotype-directed approach targeting mutated c-KIT with imatinib in melanoma yielded equally impressive results.[17]

In summary, while unselected molecular therapy in melanoma has yielded largely disappointing results, the most recent examples of genotype-directed targeted therapy in melanoma including PLX-4032 targeting activating BRAFV600 mutations and imatinib targeting c-KIT mutations, have yielded very promising results and ask together in light of the paucity of other treatment options for the rapid identification of additional targets and treatment strategies. Targeting melanoma harboring activating somatic mutations of the Erbb4 receptor with the dual tyrosine kinase inhibitor lapatinib might yield similar success as targeting activating EGFR receptor mutations in lung cancer with erlotinib or gefitinib, and might increase the

armentarium of meaningful therapeutic strategies in this disease.

2.2 Lapatinib

Mechanism of Action

Lapatinib is an allosteric, orally administered small-molecule tyrosine kinase inhibitor that targets both EGFR and Erbb2/HER2 of the Erb growth factor receptor family.[34, 35] It binds in a reversible, competitive way to the ATP-binding cleft of the C-terminal kinase domain of the receptor preventing transphosphorylation and activation of the bound co-receptor.[36, 37] Non-phosphorylated Erb receptors are not able to initiate downstream signaling either via binding to the p85 subunit of PI3K leading to activation of the PI3K-Akt pathway, nor through binding of the adaptor proteins SOS and GAP proteins and signal transduction via the MAPK pathway.[38, 39] Members of the Erbb family are structurally very similar membrane-spanning tyrosine kinase receptors composed of an extracellular domain subdivided into 4 subdomains, an α-helical transmembrane segment, and an intracellular protein kinase domain (Fig. 1).[39] Erbb receptor activation requires dimerization of two Erbb molecules which normally exist as inactive monomers. Ligand binding induces the release of intramolecular bonds ('autoinhibition') between the extracellular domains II and IV leading to a conformational twist which exposes the dimerization domain in subunit II of the receptor.[39] Pairing with another receptor leads to activation of the kinase domain, phosphorylation of the other receptor, and consequently binding and activation of downstream proteins (Fig. 1).[39] In general, the nature of the activating ligand and the different homo- and heterodimer pairings are the major determinants of which of the various downstream targets are activated. Fig. 2 depicts Erb-mediated downstream signaling via the MAPK kinase and PI3K-Akt pathways.

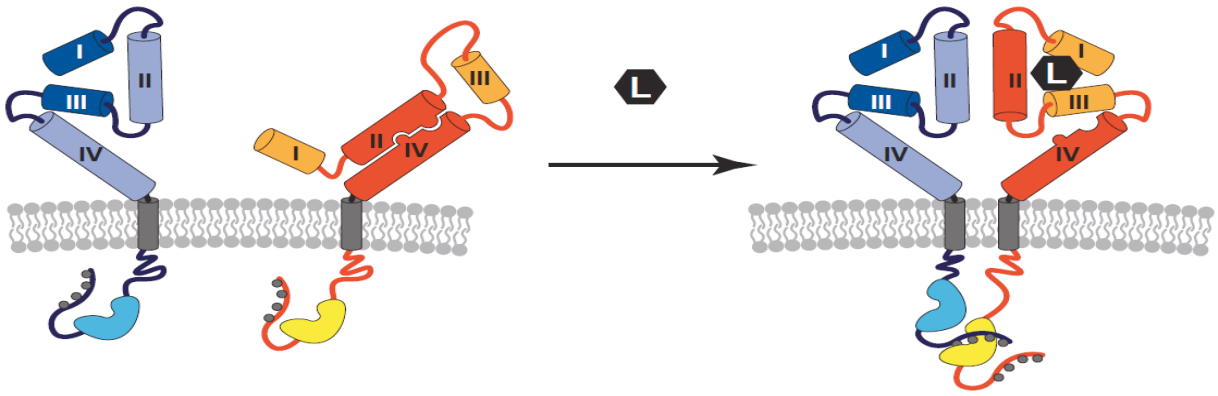


Fig 1. Ligand activation of Erb family receptors. Ligand-binding to subregions I and III of the ectodomain of the closed, 'thethered' from of the receptor prompts a conformational 'switch' of the molecule characterized by the formation of a ligand-binding pocket formed by subregions I,II, and III and a 180° twist and exposure of the dimerization region of subdomain II as a prerequisite for dimerization and signal transduction activation (from Rudloff U, Samuels Y. Cell Cycle 2010)

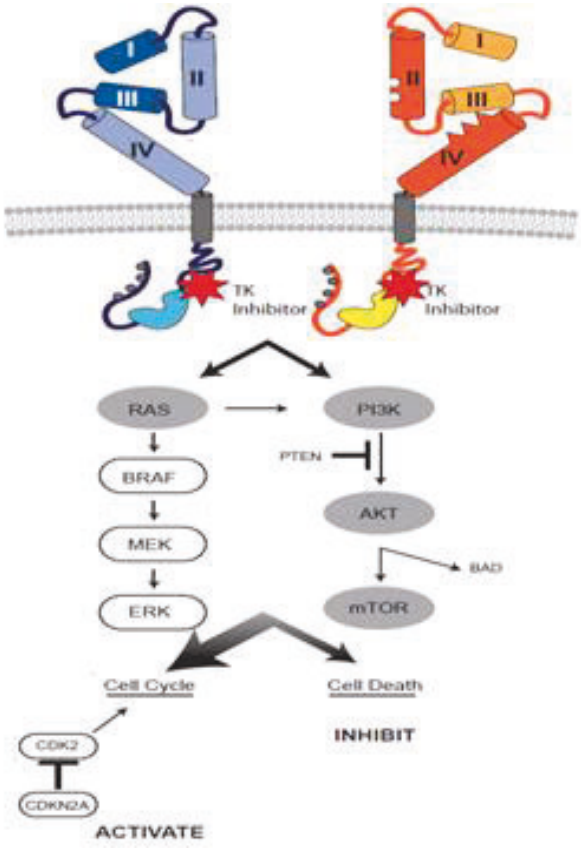


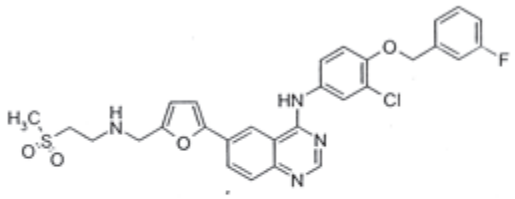
Fig. 2 Post-receptor signal transduction pathways of Erb-receptor mediated signal transductions occurs mainly via the PI3K-Akt and MAPK kinase pathways (Erbb-mediated STAT signaling not shown) (Rudloff U, Samuels Y. Cell Cycle 2010)

Activating somatic mutations of Erb receptors (e.g. L858R or del747 mutations of the kinase domain of the EGFR receptor in lung cancer) or amplification of the Erb2/HER2 receptor in breast cancer lead to abnormal, constitutive Erb receptor signaling, activation of mitogenic pathways, and abnormal cellular proliferation.[40-42] When Erb receptors are affected by somatic mutations, Erbb family members including EGFR, HER2, ErBB3, or ErBB4 are able to drive the growth of the malignancy through increased autophosphorylation and downstream signaling following dysregulated homo- and heterodimerization or constitutive activation of the kinase domain.[40-42] These tumors gain their growth advantage from abnormal Erb signaling having become 'addicted' to aberrant Erb signaling. Such 'oncogene addiction' has been shown to be the the prime model for targeted therapy, like the use

of the tyrosine kinase inhibitors erlotinib and gefitinib in lung cancer harboring EGFR mutations L858R and del747. This success now provides a strong rationale for the use of lapatinib in melanoma targeting activating somatic mutations of the ERBB4 receptor.[39, 42]

In addition to its excellent activity against EGFR and ERBB2/HER2, lapatinib also has activity against ERBB4.[34] Lapatinib binds to an inactive form of the ERBB4 kinase in a mode equivalent to its interaction with the EGFR receptor, a finding which is also supported by the fact that all *ERBB4* residues contacted by lapatinib at its binding site are conserved across multiple species in the EGFR and ERBB2/HER2 receptor.[37] Table 1 lists the inhibitory concentrations (IC50s) for the EGFR, ERBB2/HER2, ERBB4 receptor, as well as a large panel of kinases unrelated to the Erb receptor family. Lapatinib is highly selective for EGFR and HER2 enzymes, with 50% inhibitory concentrations (IC50) of 10.8 nmol/L and 9.2 nmol/L, respectively.[34] The additional kinases tested were not inhibited until IC50 values reached >3,500 nmol/L, with the exception of ERBB4, for which the IC50 was 347 nmol/L. When IC50s for ERBB4 inhibition by lapatinib were determined in various cell culture models, the IC50 *in vivo* (367 nM) were nearly identical to the IC50s predicted from the *in vitro* data and confirmed the high selectivity of lapatinib for the Erb receptor family, including ERBB4, compared to other protein kinases (Table 1).[34]

Table 1. Structure and enzyme inhibitory activity (IC50) of lapatinib (GW2016) (from Rusnak DW. Mol Cancer Ther 2001)

GW2016

N-{3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furyl]-4-quinazolinamine

Enzyme Inhibition	
Enzyme	**IC_{50} (nM)**
EGFR	10.8 +/- 0.53
ErbB-2	9.2 +/- 0.75
ErbB-4	367 +/- 4.2
c-Src	3,500 +/- 650
c-Raf-1	> 10,000*
MEK	> 10,000*
ERK	> 10,000*
c-Fms	> 10,000*
CDK1	> 10,000
CDK2	> 10,000
p38	> 10,000
Tie-2	> 10,000*
VEGFR-2	> 10,000

Pharmacokinetics

A number of phase I trials evaluating the pharmacokinetics of lapatinib have been conducted. In general, lapatinib is well tolerated without the need for dose adjustment up to 1,800 mg once daily and 500 mg twice daily.[43-48]

Lapatinib serum concentrations increase

- Proportionally with increase in daily dose
- Repeat dosing
- When taken with food, in particular high-fat meals

The pharmacokinetic properties of lapatinib for once daily and twice daily dosing are summarized in Table 2 and discussed in the following paragraphs.

Dose	Cmax (mg/L)*		Cmin (mg/L)*		AUCτ (h*mg/L)*	
	Geo. Mean†	Mean (SD)‡	Geo. Mean†	Mean (SD)‡	Geo. mean†	Mean (SD)‡
175mg QD (*n* = 3)	0.37	0.41 (0.25)	0.17	0.17 (0.05)	5.48	5.86 (2.74)
375mg QD (*n* = 3)	0.41	0.44 (0.21)	0.19	0.21 (0.13)	5.74	6.23 (3.20)
675mg QD (*n* = 4)	1.06	1.14 (0.43)	0.3	0.35 (0.18)	13.7	14.34 (5.04)
900mg QD (*n* = 4)	1.05	1.06 (0.19)	0.31	0.33 (0.10)	12.7	12.8 (1.94)
1,200 mg QD (*n* = 5)	1.39	1.55 (0.87)	0.4	0.48 (0.31)	17.3	20.0 (12.3)
1,600 mg QD (*n* = 4)	1.92	1.97 (0.58)	0.45	0.45 (0.08)	22.7	23.0 (4.1)
1,800 mg QD (*n* = 9)	1.89	2.02 (0.78)	0.74	0.82 (0.41)	27.7	29.6 (11.8)
500mg BID (*n* = 6)	2.2	2.37 (0.87)	0.96	1.10 (0.58)	18.1	19.6 (8.2)
750mg BID (*n* = 6)	1.87	2.11 (0.90)	0.92	1.15 (0.76)	15.4	18.0 (9.4)
900mg BID (*n* = 5)	3.27	3.57 (1.57)	1.77	2.04 (1.10)	28.5	31.5 (14.5)
1,250 mg fasted	0.76	0.91 (0.52)	—	—	9.07	10.7 (6.4)
1,250 mg fed	2.44	2.54 (0.84)	—	—	27.3	29.5 (12.0)

*Day 14 steady-state data for all but fasted/fed (single dose).
†Geometric mean.
‡Arithmetic mean (SD).

Table 2. Summary of pharmacokinetic data on different dosing regimens for lapatinib (from Burris HA. Clin Cancer Res 2009)

At the dose of 1,250mg daily, steady state geometric mean (95% confidence interval) values of Cmax were 2.43 mcg/mL (1.57 to 3.77 mcg/mL) and AUC were 36.2 mcg hr/mL (23.4 to 56 mcg.hr/mL).[45, 49]

Absorption

On administration of doses of 25, 100, or 175 mg, oral absorption is delayed by ~30 minutes and Tmax is 3 to 4 hours.[43] Time-dependent increases in Cmax and AUC are detected at the higher doses (100 and 175 mg) but not at the lower dose.
At the FDA-approved dose of 1,250 mg/d, the steady-state Cmax and AUC are 2.54 µg/mL and 36.2 µg h/mL, respectively.[50, 51] Administering lapatinib in divided daily doses increased the steady-state AUC ~2-fold compared with the same total dose administered once daily (Fig. 3).[45]

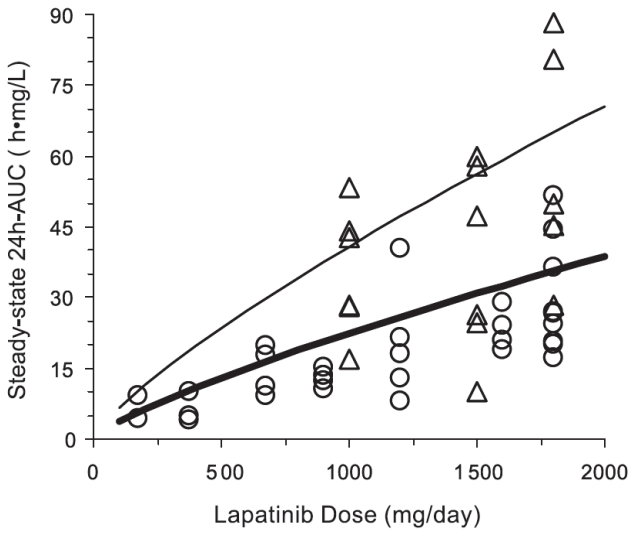


Fig 3. Steady state 24-h AUC for once daily dosing (fat curve; o) and twice daily dosing (thin curve; Δ) of lapatinib (from Burris HA. Clin Cancer Res 2009)

With multiple daily dosing of lapatinib, steady state was achieved within 6 to 7 days.[45] Systemic exposure to lapatinib is increased when the drug was administered with food, the greatest effect occurring with a high-fat meal.[52] The AUC was increased by 325% and 167% when taken with high- and low-fat meals, respectively.[52] However, while administration with food increased bioavailability of lapatinib, there was also increased variability in drug concentration potentially leading to increased toxicity. [52]

Distribution

At concentrations of 1 μmol/L, lapatinib is highly bound (>99%) to albumin and to α-1 acid glycoprotein.[52] The volume of distribution (Vd/L) of the terminal phase of lapatinib is >2,200 L, indicating good drug distribution.[51, 52]

Metabolism

Lapatinib is primarily metabolized to oxidated metabolites by the cytochrome P450 (CYP) 3A4, 3A5, 2C19, and 2C8 isozymes.[44] Approximately 70% of its metabolism is through CYP3A4. No metabolite constitutes >15% of recovered drug. One metabolite (GW690006) remains active against EGFR but not HER2, whereas other metabolites appear to be inactive.[51]

Elimination

Lapatinib is primarily eliminated hepatically, with 27% of an oral dose recovered in feces and <2% recovered in the urine.[50] The elimination t 1/2 is 14.2 hours in single-dose studies and ~24 hours with repeated dosing (as a result of drug accumulation).[50, 51]

Drug interactions

Lapatinib is a major substrate for and inhibitor of CYP3A4 and an inhibitor of CYP2C8. Ketoconazole, a CYP3A4 inhibitor, increases the AUC of lapatinib 3.6-

fold relative to control, and the t1/2 increased 1.7-fold relative to control.[50, 51] In contrast, carbamazepine, a CYP3A4 inducer, decreased the AUC of lapatinib by ~75%.[50, 51]

Maximum Clinically Achievable Concentration of Lapatinib

Based on the available PK data for lapatinib, a dosing regimen of 500 mg twice daily provides the highest Cmax and Cmin without prohibitive GI toxicity.[45] Calculating Cmax and Cmin for both 1,200 mg once daily and 500 mg twice daily, the maximally achievable concentration in humans for lapatinib is 4.07×10^{-6} mol/L. This information is based on

Assumptions: Tmax = 3.5hrs

$$C = C_0 e^{-kt}$$

$$t_{\frac{1}{2}} = \frac{\ln 0.5}{-k} = \frac{0.693}{k}$$

1200mg PO q 24hrs:

Cmax: 1.55mg/L

Cmin: 0.48mg/L

$$\frac{0.48mg}{L} = (\frac{1.55mg}{L})\, e^{-k(21.5hrs)}$$

$$k = 0.0542$$

$$t_{\frac{1}{2}} = 12.7hrs$$

500mg PO q 12hrs:

Cmax: 2.37mg/L

Cmin: 1.1mg/L

$$\frac{1.1mg}{L} = (\frac{2.37mg}{L})\, e^{-k(3.5hrs)}$$

$$k = 0.0903$$

$$t_{\frac{1}{2}} = 7.7hrs$$

and the known molecular weight of lapatinib of 943.5.

Lapatinib in Phase 2 and 3 Studies with Advanced or Metastatic Breast Cancer

In the sentinel, randomized, open-label phase 2 study of lapatinib monotherapy as first-line treatment in patients with HER2-positive advanced or metastatic breast cancer, patients were randomized to receive oral lapatinib either as 1,500 mg QD (n = 69) or 500 mg BID (n = 69).[53] Overall, there was a 24% response rate (CR + PR) and a clinical benefit (CR, PR, + SD >24 weeks) of 31%, with no significant differences in these outcomes between treatment groups. Most responses (32/33) occurred by week 12, with a median time to response of 7.9 weeks. The responses were durable, with a median duration of response of 28.4 weeks and a 6-month PFS of 43%. This study showed that lapatinib in breast cancer has clinical efficacy as first-line treatment.[53]

In the international, multicenter, open label phase 3 trial leading to regulatory approval of lapatinib in HER2-positive metastatic breast cancer that is refractory to regimens including an anthracycline, a taxane, and trastuzumab, patients were randomly assigned in a 1:1 ratio to receive oral lapatinib 1,250 mg QD on an ongoing basis and oral capecitabine 2000 mg/m^2 given in 2 divided doses on days 1 through 14 of a 21-day cycle, or monotherapy with capecitabine 2,500 mg/m^2 given in 2 divided doses on days 1 through 14 of a 21-day cycle.[54] The primary end point, TTP, was defined as the time from randomization to tumor progression or death related to breast cancer. After independent review, the interim analysis of TTP met the prespecified criteria for early reporting based on the superiority of lapatinib + capecitabine relative to capecitabine monotherapy.[54] There were 49 disease-progression events in the combination therapy group and 72 in the monotherapy group, and the median TTP was 8.4 and 4.4 months, respectively. The hazard ratio (HR) for TTP with combination therapy was 0.49 (95% CI, 0.34-0.71; P < 0.001). At the time of the interim analysis, there were no differences in OS between the groups, and 22% of patients in both groups had died. The updated analysis included 82 disease-progression events with the combination of lapatinib + capecitabine and 102 events with capecitabine monotherapy confirmed these findings.[55] The median TTP for combination therapy was 6.2 months, compared with 4.3 months for capecitabine alone (HR = 0.57; 95% CI, 0.43-0.77; P < 0.001), indicating a 43% reduction in the relative risk of progression with the addition of lapatinib to capecitabine. Similarly, there was an improvement in PFS (HR = 0.55; 95% CI, 0.40-0.74; P < 0.001), indicating a 45% relative risk reduction in PFS with combination therapy. Although there was a reported improvement in OS with combination therapy (HR = 0.78), the difference did not reach statistical significance. Appendix A summarizes the outcome of clinical trials of lapatinib in advanced or metastatic breast cancer.[49]

For phase II drugs against solid organ tumors, designs with (p1=targeted response rate; p0=low response rate) equal to (0.05; 0.20), (0.05; 0.25), or (0.10; 0.30) are most commonly used. Even when done in a genotype-directed fashion, molecular therapy trials have often only response rates of 25 – 30% in the metastatic setting when administering the agent to heavily pretreated patients. Additionally, as listed above, response rates of advanced or metastatic breast cancer to lapatinib have been reported in the 4.3 – 35.1% range (Appendix A). Thus, while p1 – p0 = 0.15 is reaching the smallest difference considered clinically interpretable, we are aiming to target 30% and hoping to rule out 10% as our response rate.

Toxicity Profile of Lapatinib

Similar to other tyrosine kinase inhibitors, lapatinib is well tolerated. The most common clinical toxicities of all grades (occurring in ≥ 10%) associated with lapatinib in the sentinel phase 1 and 2 study of lapatinib monotherapy were diarrhea, rash, pruritus, and nausea (diarrhea (46%) and rash (32%)).[45, 49, 54] Diarrhea events were mainly grade 1 or 2, as were rash events.[49] The incidence of adverse events (AEs) considered by investigators to be related to lapatinib was equal between the

treatment regimens of 1,500 mg daily and 500 mg twice daily, affecting 71% of patients overall.[60] These events were also mainly grade 1 or 2 in toxicity (60%); only 9%, less than 1% of all patients, were reported to be grade 3, 4, and 5. The most frequently reported AEs related to lapatinib monotherapy, including diarrhea (36%), rash (27%), pruritus (18%), and nausea (10%), are listed in Table 3. All other lapatinib-related AEs occurred in less than 10% of patients.[53]

	Dosing Regimen					
	1,500 mg Once Daily (n = 69)		500 mg Twice Daily (n = 69)		All Patients (N = 138)	
Adverse Event*	No.	%	No.	%	No.	%
Diarrhea	24	35	25	36	49	36
Grade 1-2	23	33	22	32	45	33
Grade 3	1	1	3	4	4	3
Rash	19	28	18	26	37	27
Grade 1-2	19	28	17	25	36	26
Grade 3	0	0	1	1	1	1
Pruritus	14	20	11	16	25	18
Grade 1-2	14	20	11	16	25	18
Grade 3	0	0	0	0	0	0
Nausea	9	13	5	7	14	10
Grade 1-2	9	13	4	6	13	9
Grade 3	0	0	1	1	1	1

*No grade 4 adverse events occurred for these conditions.

Table 3. Patients with drug-related adverse events that occurred in 10% of patients receiving lapatinib monotherapy (from Gomez HL. JCO 2008)

Similar results were observed when lapatinib was given in combination with capecitabine where diarrhea (65%), hand-foot syndrome (53 %), nausea (44%), rash (29%), and fatigue (24%) occurred most commonly.[54, 55] Discontinuation rates of lapatinib in both the combination and monotherapy group occurred in 14% of the cases and did not differ.[55] Dose interruptions and adjustments due to AEs did also not differ between groups. Diarrhea and rash occurred more occurred more frequently in the combination-therapy group than in the monotherapy group (65% vs 34%, and 29% vs 14%, respectively), however, the differences in both diarrhea and rash were the result of increases in grade 1 adverse events with no statistically significant difference in higher-grade toxicities.

GI Toxicity

In the sentinel phase 1 trial of lapatinib, the incidence of diarrhea has been found to increase at higher doses, not at higher serum concentrations, suggesting that this adverse effect is the result of unabsorbed drug.[44] The development of rash did not appear to be dose or serum related.[44, 45]

In the twice daily dosing group, grade 3 diarrhea (total of 17%) was the limiting toxicity.[60] None in the 500 mg twice daily group required dose reductions due to diarrhea. However, in the 900 mg twice daily and 750 mg twice daily group, 33 percent of patients required dose modifications.[60] The frequency of diarrhea as the most common dose-limiting toxicity (DLT) of lapatinib following administration once (QD) and twice (BID) daily doses is shown in Fig. 4 (from Burris HA. Clin Cancer Res 2009).

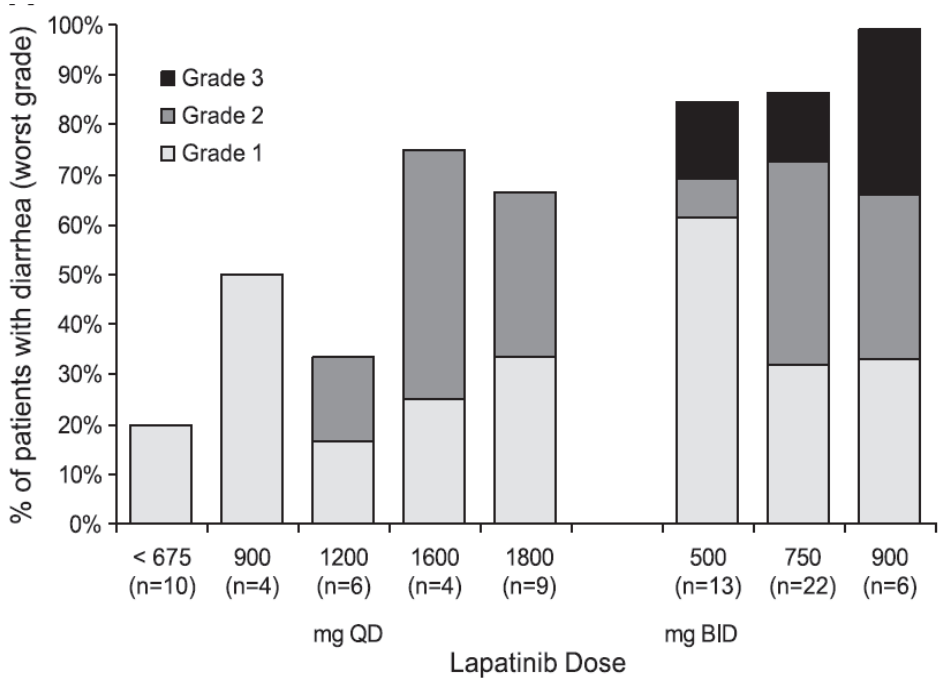


Fig. 4 Grade 1, 2, and 3 diarrhea as the most common adverse event in relation to different doses and dosing schedules (from Burris HA. Clin Cancer Res 2009)

In general, diarrhea events with lapatinib are in the majority of cases of grade 1 and 2 severity. No fatal lapatinib-associated diarrhea events have been reported. Lapatinib-related diarrhea occurs early usually within the first two weeks of treatment.[60] The median duration of diarrhea with lapatinib was 5 days, with 85% of patients requiring no dose adjustment or interruption, and only 2% discontinuing therapy due to diarrhea.[60] In general, severe diarrhea did not occur with lapatinib-containing regimens when proactive monitoring and appropriate intervention was instituted in a timely manner.

Dermatologic Toxicity

The second most frequently reported adverse events associated with lapatinib consist of dermatologic toxicities. Similar to lapatinib-related diarrhea, these are most commonly toxicities of grade 1 and 2 severity.[61] These include in decreasing order hand-foot syndrome (palmar plantar erythrodysesthesia), rash, hair disorder, dry skin, pruritus/urticaria, skin disorder, skin infection, and nail disorder. In patients

receiving lapatinib monotherapy, 55% experienced grade 1 or 2 dermatologic events; no grade 4 events were observed.[53] Rash was the most common dermatologic event (43%). Dermatologic events typically developed early (within days 1-14), lasted a median of 29 days, and did not require any intervention or lapatinib dose reduction, interruption, or discontinuation.[54, 61]

Cardiac Toxicity

In a comprehensive review of 3,689 patients enrolled in phase 1-3 trials of lapatinib monotherapy and combination therapy in breast cancer a search for cardiotoxicity was performed by using either grade 3 or 4 toxicities according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) or asymptomatic decreases in LVEF >20% from baseline or below the institutional normal.[62] The cardiac event rate was reported as 1.6% (n = 60); the event was not preceded by symptoms in 53 of these 60 patients.[62] In 88% (n = 35) of the 40 patients the decrease in ventricular function improved or resolved spontaneously. The decrease was rarely severe (0.2%). These preliminary data indicate no incremental risk of cardiac toxicity with the addition of lapatinib, although further studies of the cardiac tolerability of this combination are needed.

Hepatic Toxicity

Since lapatinib is mainly eliminated hepatically, dose adjustments are advised in patients with hepatic dysfunction.[51] In a small study in patients with Child-Pugh class B and C liver impairment (excluded from this study), AUC after one single dose of 100 mg lapatinib increased more than three-fold.[50] Dose reductions to 500 mg or 750 mg daily have been suggested.

2.3 Rationale

Mutations of the Growth Factor Receptor Kinase 4 (*ERBB4*) are Common in Metastatic Melanoma

One of the most promising aspects of cancer treatment is genotype-directed therapeutics targeted at constitutively activated signal transduction pathways.[15] A recent high-throughput sequencing effort of the entire protein tyrosine kinase gene family in a large tumor bank of metastatic melanoma tissues identified several novel promising targets in this disease.[63] Sequence analysis of 593 exons of 89 genes of 79 melanoma samples (~ 12Mb) identified 19 genes with somatic mutations within the kinase domains of protein tyrosine kinases.[63] In total, 19 percent of melanoma samples contained somatic gene mutations of the ERBB4 receptor, making it together with Braf, TP53, CDKN2A, and PTEN one of the most commonly mutated genes in melanoma.

Increased activity of Erb receptors due to activating somatic gene mutations or amplifications of the HER2 gene has been found in several other histologies like breast, lung, and colon cancers, among others.[64, 65] Targeted therapy with trastuzumab (Heceptin), erlotinib, or gefitinib against Erb or EGFR mediated cell-signaling in breast, pancreas, or lung cancer, to name a few, has recently found FDA

approval and has now an established role in the treatment of these malignancies.[66-69] Mutated *ERBB4* appears now to be a similarly attractive target for molecular therapy in melanoma:

Mutations of *ERBB4* are Oncogenic in Melanoma

ERBB4 mutants show increased autophosphorylation and activation of the ERBB4 receptor

To show that mutations of *ERBB4* identified in melanoma patients have increased kinase activity and increased activation of the ERBB4 receptor, wild type (WT) *ERBB4* or seven mutants (E317K, E452K, E542K, R544W, E563K, E836K, E872K) found in the sequencing screen and shown in Fig. 4 were stably expressed in both HK293T cells and human melanoma cell lines. Compared to WT *ERBB4* expressing cell lines, all the missense mutants showed a marked increase in total phosphorylation of the receptor, and increased tyrosine phosphorylation of the *ERBB4* mutants correlated with increased kinase activity and activation of *ERBB4*.

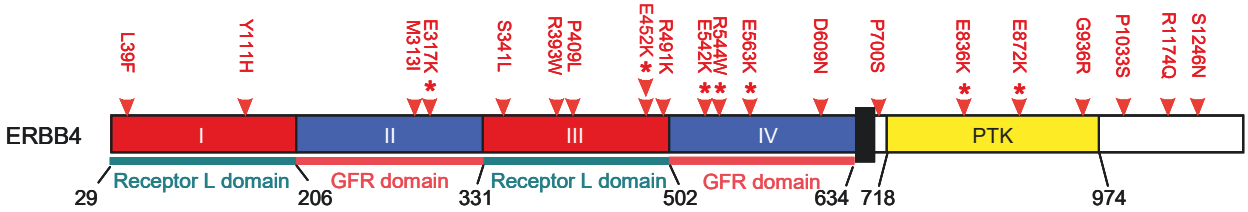


Fig. 5 Distribution of mutations in *ERBB4* in metastatic melanoma. Red stars indicate ERBB4 mutants used in subsequent analysis (from Prickett TD. Nat Genet 2009)

Mutated ERBB4 leads to increased activation of the Akt pathway

ERBB4 is known to activate several downstream signaling pathways including the extracellular signal-regulated kinase 1 (ERK1) and ERK2 pathway and the AKT pathway. Both pathways are known to play pivotal roles in the process of malignant transformation in the development of various cancers including melanoma.[21, 70] Phosphoimmunoblot experiments in transfected melanoma cells demonstrated that the identified mutations in the *ERBB4* receptor lead to activation of the Akt pathway in melanoma. Downstream signaling of mutated *ERBB4* is nearly exclusively mediated via the Akt pathway with no effect on the MAPK pathway.

Mutations of the ERBB4 receptor are oncogenic in melanoma

To determine that the ERBB4 variants are transforming, NIH 3T3 cells were transiently transfected with vector, WT *ERBB4*, the seven constitutively active *ERBB4* mutants described above, or oncogenic K-RasG12V. All *ERBB4* mutants transformed NIH 3T3 cells more efficiently than WT *ERBB4*. Strikingly, the transformation ability of the *ERBB4* mutations was similar to oncogenic K-Ras G12V, one of the most potent oncogenes. The transformation abilities of mutant

ERBB4 are shown in Fig. 6 by significantly increased anchorage-independent growth of NIH 3T3 and melanoma SK-Mel-2 cells transfected with mutant *ERBB4.*

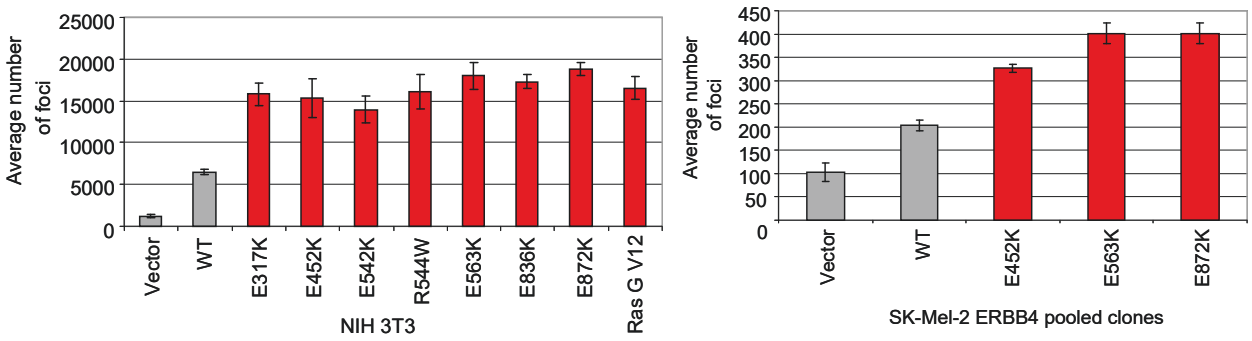


Fig. 6 Mutant *ERBB4* induces cell transformation and anchorage-independent growth in NIH3T3 and melanoma SK-Mel-2 cells (Prickett TD. Nat Genet 2009)

In order to show that melanoma cells harboring endogenous *ERBB4* mutations are dependent on *ERBB4* signaling for proliferation, short hairpin RNA (shRNA) was used to stably knockdown ERBB4 protein levels in melanoma lines harboring either WT or mutant *ERBB4*. Three unique shRNA constructs targeting ERBB4 had minimal effect on the proliferation of cells expressing WT receptor but significantly reduced the growth of melanoma lines containing mutant *ERBB4* (Fig. 7). Evaluation of the effects of *ERBB4* knockdown on downstream signaling pathways revealed that down-regulation of ERBB4 in cells harboring mutant versions of the gene reduces levels of endogenous, phosphorylated Akt, but not of phosphorylated ERK.

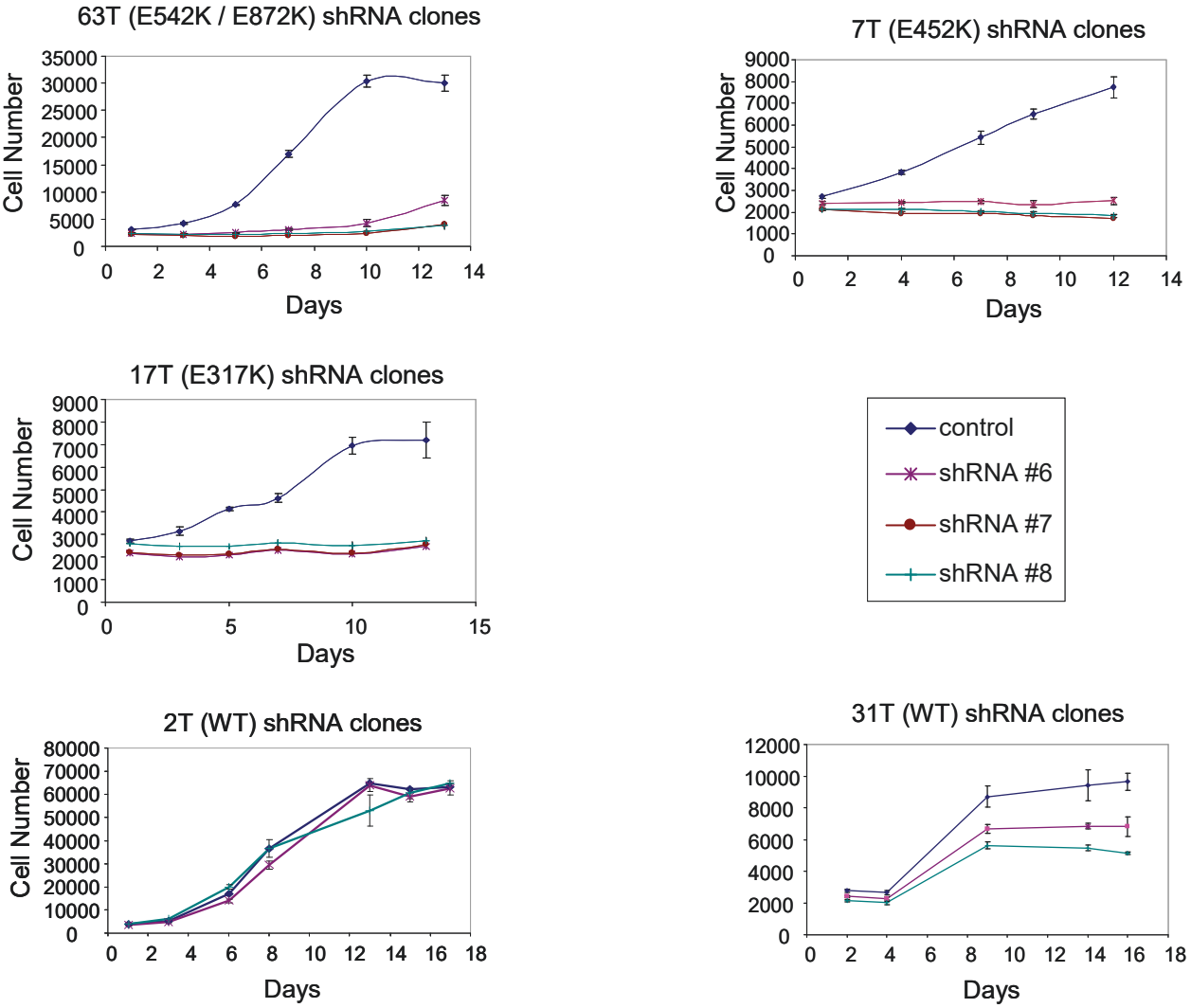


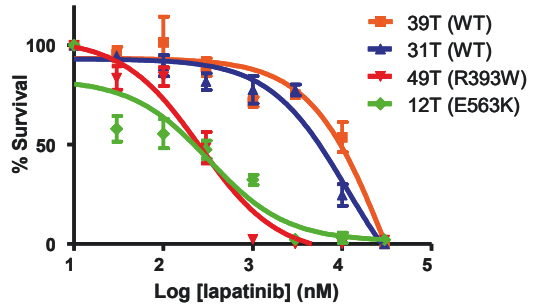
Fig. 7 Expression of mutant *ERBB4* provides an essential cell survival signal in melanoma. Knockdown of endogenous, mutant *ERBB4* with shRNA abolishes cell growth in metastatic melanoma (Prickett TD. Nat Genet 2009)

In summary, mutant *ERBB4* is essential for growth of melanoma cells harboring these mutations, and 'oncogene addiction' in these melanomas is mediated by activation of the Akt pathway.

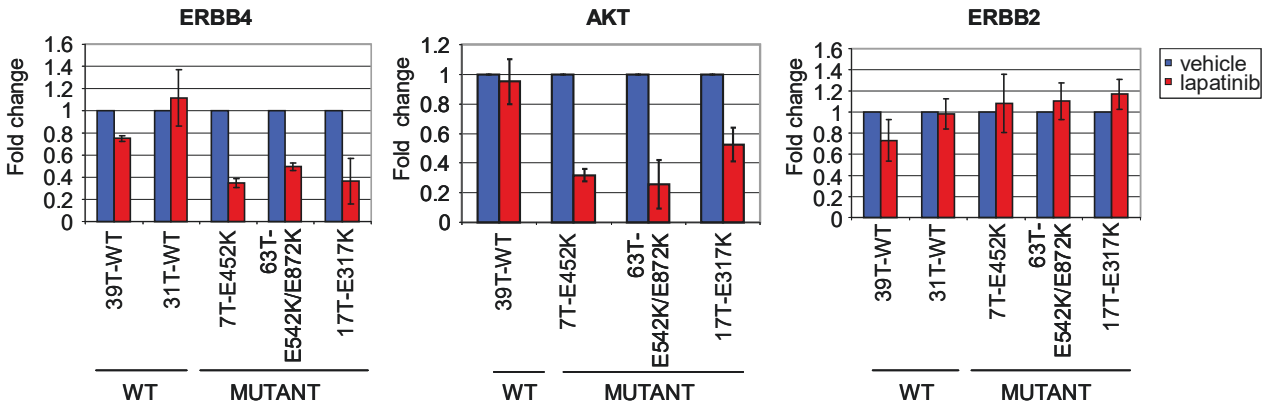
Lapatinib effectively inhibits ERBB4 mediated cell signaling in vitro and in vivo in metastatic melanoma

Exposure of melanoma cells to lapatinib resulted in reduced cell proliferation to a significantly greater extent in cells containing *ERBB4* mutations than in cells containing endogenous WT *ERBB4* (Fig. 8). An IC50 calculation of this inhibition revealed that melanoma cells harboring *ERBB4* mutations were 10-250 fold more

sensitive to lapatinib than cells with WT receptor.



Cell line (mutation)	IC50 (nM)	Std Error (+/- nM)
68T (splice site)	131.8	1.97
49T (R393W)	259.6	3.27
12T (E563K)	324.6	4.87
17T (E317K)	513.8	6.36
7T (E452K)	1365	19.3
71T (L39F, S1246N)	1446	19.5
63T (M313I, E542K, E872K, R1174Q)	3213	40.5
31T (WT)	11370	157
93T (WT)	31860	523
2T (WT)	32980	861
39T (WT)	34050	661



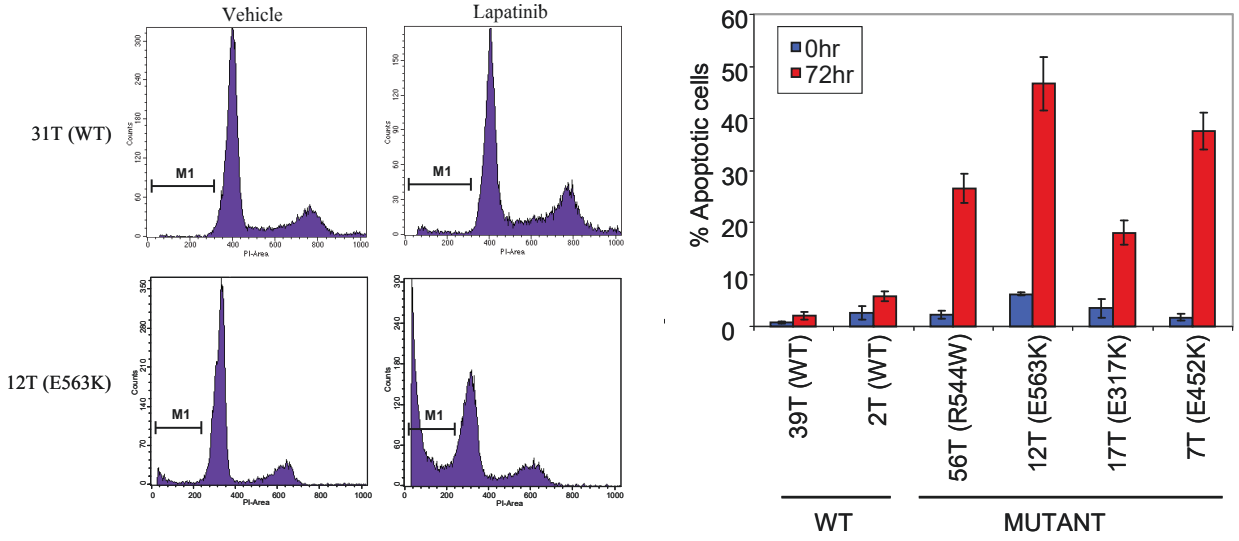


Fig. 8 Melanoma lines expressing *ERBB4* mutants are sensitive to *ERBB4* inhibition by lapatinib. Representative dose response curves for *ERBB4* WT and *ERBB4* mutants, the inhibition of Akt signaling by lapatinib, and the induction of apoptosis by lapatinib in *ERBB4* mutants is shown (Prickett TD. Nat Genet 2009)

It must be noted that the observed reduced proliferation occurred in cells harboring BRAF, NRAS, ARAF or CRAF mutations in addition to the *ERBB4* mutations reaffirming the 'addictive oncogenic nature' of *ERBB4* mutations in melanoma.[63] Table 4 shows that sensitivity of melanomas harboring *ERBB4* mutations to lapatinib was not impacted by the presence of concomitant BRAF or NRAS mutations as e.g. the most sensitive mutants (68T with IC50 value of 131.8nmol and 49T IC50 259.6nmol; Fig 8) both harbored concomitant BRAFV600E mutations.

Table 4 The presence of concomitant RAF and RAS mutations in melanoma samples harboring Erbb4 mutations.

Sample	ERBB4	BRAF	NRAS	ARAF	CRAF	HRAS	KRAS
7T	E452K	wt	Q61R	wt	wt	wt	wt
12T	E563K	wt	Q61Q/R	wt	wt	wt	wt
17T	E317K	wt	Q61Q/K	wt	wt	wt	wt
31T	wt	wt	wt	wt	wt	wt	wt
34T	R491K	V600V/E	wt	wt	T362T/A	wt	wt
39T	wt	wt	wt	wt	wt	wt	wt
49T	R393R/W	V600V/E	wt	wt	wt	wt	wt
55T	E452K	V600V/E	wt	P216S, P254L	wt	wt	wt
56T	R544R/W	V600V/E	wt	wt	wt	wt	wt
63T	E542K, E872K	wt	Q61Q/K	wt	wt	wt	wt
68T	Splice Site / LOH	V600V/E	wt	wt	wt	wt	wt
71T	L39L/F, S1246S/N	V600V/M, V600V/E	wt	wt	wt	wt	wt
86T	E836E/K	V600V/E	wt	wt	wt	wt	wt
93T	wt	wt	wt	A345A/G	wt	wt	wt

In this study, patients with melanoma harboring more than 2 mutations in the *ERBB4* gene will be excluded because of increased resistance to lapatinib due to synergy of activating mutations. As the number of mutations that can activate ERBB4 is particularly large, due to the large and heterogenous number of residues that can lead to either stimulation of activating domains or abrogation of negative regulatory domains, the presence of multiple (>2) mutations has been found to be associated with increased autophosphorylation, receptor activation, downstream signaling, and lapatinib resistance (please see table in Figure 8 above).

In summary, ERBB4 represents a near ideal target for personalized treatment of metastatic melanoma. Unlike the oncogene Ras, the near exclusive vertical signal

transduction of mutated *ERBB4* in melanoma via the Akt pathway are ideal prerequisites for successful abolition of its oncogenic potential and for cancer treatment in this tumor in general.

2.4 Correlative Studies Background

Genetic Fingerprinting of Melanoma Samples

Response to lapatinib in patients with metastatic melanoma harboring *ERBB4* mutations is determined by

(1) the intrinsic type of the individual *ERBB4* mutation and its impact on receptor activation via mechanisms like increased ligand binding, increased receptor dimerization, increased ATP binding, and increased transphosphorylation of the paired receptor, and

(2) extrinsically, by other genetic alterations of the tumor and their influence on aberrant cell signaling.[39]

With respect to activating somatic mutations of the ERBB4 receptor, major oncogenic events in melanoma like activation of the MAPK kinase pathway through activating mutations in the BRAF kinase (present in more than 60% of melanomas), activation of the PI3K-Akt pathway through PTEN loss (20 – 50% of melanomas), or increased cell cycle progression via loss of the INK4A (p16) locus do not occur in an exclusive fashion and might therefore facilitate the activation of escape pathways following Erbb4 inhibition with lapatinib.[14, 71]

Therefore, knowledge of other major genetic alterations in these melanoma samples will aid in

1. Identifying tumors less responsive and/or resistant to lapatinib treatment
2. The design of combination molecular therapy by targeting escape pathways, as the majority of these pathways are now amenable to pharmacological manipulation

In addition to all 28 exons of ERBB4 (please see Appendix B for ERBB4 primers), exons of most common oncogenes / tumor suppressor genes affected most frequently by genetic alterations in cutaneous melanoma will be analyzed by Sanger sequencing (please see Section 5.4 and Appendices H-J).

Pharmacokinetic Analysis of Patient Blood Samples

In order to better characterize the lapatinib bioavailability when administered at the 500 mg BID dose when taken one hour before or one hour after a major meal, PK samples will be collected. PK analysis will be performed as per section 5.4.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed stage IV cutaneous melanoma.

3.1.2 Patients must have measurable disease defined by RECIST 1.1. Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded). Each lesion must be > 10 mm when measured by CT, MRI or caliper measurement by clinical exam; or > 20 mm when measured by chest x-ray. Lymph nodes must be > 15 mm in short axis when measured by CT or MRI. See Section 10 for the evaluation of measurable disease.

3.1.3 Patients must have no more than two oncogenic somatic *ERBB4* mutations detected in one of the 28 exons of the *ERBB4* gene analyzed from the tumor which is not a small nucleotide polymorphism (SNP) as determined by sequence analysis of matched normal DNA from any specimen obtained from the individual at the participating clinical sites. Sequence analysis will be performed at the NIH Clinical Molecular Profiling Core, a CLIA-certified laboratory.
Note: Patients with 3 or more mutations in their ERBB4 gene may have an increased resistance to lapatinib and are not eligible for lapatinib treatment

3.1.4 Patients must not have had chemotherapy, molecular therapy with B-RAF, MEK, or c-kit inhibitors, hormonal therapy, radiation therapy, or biological therapy for at least 4 weeks prior to starting study medication. Patients who received mitomycin C, nitrosoureas, anti-CTLA-4, or carboplatin must be 6 weeks from the last administration of therapy. Patients must have recovered from any acute toxicity related to prior therapy or surgery, to a grade 1 or less unless specified.

3.1.5 Patients with no more than 3 intracranial metastases, which have been definitively treated by surgery or radiation therapy may be eligible for study provided there is no evidence of active disease for at least 2 months and no requirement for anticonvulsant therapy or steroids following treatment.

3.1.6 Age ≥18 years.
Note: Because no dosing or adverse event data are currently available on the use of lapatinib in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric single-agent trials, if applicable.

3.1.7 Life expectancy of greater than 3 months.

3.1.8 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix C).

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

– absolute neutrophil count	≥1,500/mcL
– platelets	≥75,000/mcL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	≤3 x institutional upper limit of normal
– creatinine	less than or equal to institutional upper limit of normal
OR	
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.10 Patients must be willing to return to the Clinic for follow-up visits.

3.1.11 Women of childbearing potential must have a negative beta-HCG (serum or urine) within 14 days prior to study treatment and must be willing to practice effective birth control to prevent pregnancy while receiving treatment and for four months after treatment is discontinued. All males of child fathering potential must also be willing to practice effective birth control.
Note: Lapatinib is a tyrosine kinase inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with lapatinib, breastfeeding should be discontinued if the mother is treated with lapatinib. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, the patient should inform the treating physician immediately.

3.1.12 Ability to understand and the willingness to sign the Informed Consent Document.

3.1.13 Patients who agree to participate in the PK portion of the study must be able to swallow lapatinib tablets for the duration of the PK studies.

3.2 Exclusion Criteria

3.2.1 Patients may not be currently receiving any other investigational agents.

3.2.2 Patients may not have received prior treatment with tyrosine kinase inhibitors (e.g., lapatinib erlotinib, or gefitinib).

3.2.3 Patients currently receiving any medication known to induce/inhibit CYP3A4 as listed in Appendix D, which in the opinion of the principal investigator, would make the administration of study drug hazardous. *Note: patients receiving any strong or moderate CYP3A4 inhibitors will be excluded.*

3.2.4 Patients with active hepatic or biliary disease (with exception of patients with Gilbert's syndrome, asymptomatic gallstones, liver metastases or stable chronic liver disease per investigator assessment)

3.2.5 Patients with 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.

3.2.6 HIV-positive patients on antiretroviral therapy are excluded because most antiretrovirals are strong or moderate CYP3A4 inhibitors (please see 3.2.4 above).

3.2.7 Any underlying medical condition which, in the opinion of the principal investigator, will make the administration of study drug hazardous or obscure the interpretation of adverse events

3.2.8 Patients with any other concurrent malignancy, except for the following: adequately treated basal or squamous cell skin cancer, superficial bladder cancer, carcinoma in situ of the cervix, or any other cancer from which the patient has been disease-free for five (5) years or more.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. Based on the nature of the disease and the primary patient population it affects, the planned accrual targets is shown in the table below:

Accrual Targets

Ethnic Category	Sex/Gender				
	Females		Males		Total
Hispanic or Latino	1	+	1	=	2
Not Hispanic or Latino	11	+	12	=	23
Ethnic Category: Total of all subjects	12	+	13	=	25
Racial Category					
American Indian or Alaskan Native	0	+	0	=	
Asian	0	+	0	=	
Black or African American	0	+	0	=	
Native Hawaiian or other Pacific Islander	1	+	1	=	2
White	11	+	12	=	23
Racial Category: Total of all subjects	12	+	13	=	25
	(A1 = A2)		(B1 = B2)		(C1 = C2)

3.4 Research Eligibility Evaluation

All Patients

ERBB4 mutation screening

Tumor tissue will be obtained for *ERBB4* mutation analysis, which will take place at the NIH Clinical Molecular Profiling Core laboratory (Dr. P. Meltzer, NCI/NIH). In addition, a blood sample or normal tissue adjacent to the tumor biopsy will be submitted with the tumor tissue specimen and used as matched normal control. Only patients with up to two *ERBB4* somatic gene mutations on genomic sequence analysis of baseline biopsy will proceed to the treatment part of the trial. Appendix E contains the flowsheet with instructions for submission of tissue sample for *ERBB4* analysis by participating institutions. Appendix B contains a list of the validated primers for all 28 ERBB4 exons.

Patients with confirmed *ERBB4* mutations

To be completed within 4 weeks of treatment

3.4.1 Complete history and physical examination

- Height and weight, vital signs
- ECOG performance score
- Documentation of measurable disease (to be performed prior to enrollment in study)
- Documentation of all prior therapies (e.g., hormonal, surgical, radio-therapeutic and cytotoxic).

3.4.2 Medication History

- A complete medication history should be obtained prior to starting the study agents including over the counter medications, homeopathic remedies, vitamins, and alternative therapies.

3.4.3 Imaging Studies

- CT Scan, CT/PET scan, and/or other standard imaging method as determined at the time of the first evaluation (i.e., screening visit) to include oral and IV contrast of areas of known or suspected disease involvement. This includes CT, CT/PET, or MRI of the brain.
- Left Ventricular Ejection Fraction (LVEF) by ECHO.

To be completed within 2 weeks of treatment:

3.4.4 Laboratory Evaluation [baseline is to be obtained within 14 days prior to enrollment]

- CBC with differential and platelet count

- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- Urinalysis
- PT/aPTT/fibrinogen or INR
- Beta-HCG (serum or urine pregnancy test) for females of child bearing potential.

4. REGISTRATION PROCEDURES

4.1 Participating Sites

Patients will be registered by the PI designee or research nurse at the participating site within 2 weeks of the patient signing the screening consent by faxing a screening Eligibility Checklist with the appropriate sections completed to the Central Registration Office (CRO) at 301-480-0757. Once it has been determined that the patient has the *ERBB4* mutation in their tumor, a separate treatment consent and treatment eligibility checklist will be completed and faxed to the Principal Investigator, Udo Rudloff, M.D., Ph.D., or his designee for review (301-451-6933) prior to patients starting treatment. The PI or his designee will review and fax the completed treatment eligibility checklist to the CRO at 301-480-0757 for registration on to the treatment phase of the protocol. The CRO will fax confirmation of registration to the coordinating center and the site.

4.2 NCI

Patients enrolled at the NCI Clinical Center will be screened under the Surgery Branch screening protocol 99-C-0128 "Evaluation for NCI Surgery Branch Clinical Research Protocols". Registration of patients onto this study will take place within 24 hours of the patient signing the 99-C-0128 consent by faxing a completed eligibility checklist to the Central Registration Office (CRO) at 301-480-0757. Once it has been determined that the patient has the *ERBB4* mutation in their tumor, the treatment consent for protocol 8786 (NCI 11-C-0048) and treatment eligibility checklist will be completed and faxed to the Central Registration Office (CRO) at 301-480-0757.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov).

5. TREATMENT PLAN

5.1 Lapatinib Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications lapatinib

are described in Section 5.2 below. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Lapatinib will be administered as an oral dose of 500 mg twice daily (in the morning and evening). All medications are given daily for 28 days, 28 days (4 weeks) equals one cycle. The first course will consist of 28 days (one cycle). All subsequent courses will consist of 2 cycles. Up to 27 cycles may be administered (14 courses, approximately 2 years).

Patients will be instructed to take the medications at the same time each day taken one hour before or after meals. Patients will be advised not to consume grapefruit or grapefruit juice while taking the study medications. The patient will be requested to maintain a medication diary of each dose of medication and any side effects and/or adverse events the patient may experience. The medication diary will be returned to clinic staff at the end of each course.

Prior to discharge from the clinic, the research nurse will review the following information with the patient:

- The toxicities of each agent and how to treat/prevent them
- The signs and symptoms to report to the study team
- How to complete the medication self administration record (patient diary)(See Appendix F for lapatinib-specific medication diary).
- Instructions to carefully observe for signs and symptoms of AEs
- Potential risks regarding pregnancy and fetal exposure
- Study agents should never be shared

Patients will be dispensed 1 cycle of drug at a time (Section 5.3.7). At each cycle prior to drug dispensing, the toxicity information as described in section 7 will be reviewed with the patient.

5.2 Dosing Delays/Modifications

General guidelines for treatment modifications at the time of re-treatment:

Adverse Events: All adverse events in this trial will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 - a complete listing is available at the CTEP website: http://ctep.cancer.gov/forms/CTCAEv4.pdf.

Dose adjustments will be made according to the guidelines below, with dose levels defined as follows:

Dose Level (DL)	***Agent*** **Dose**
0	Lapatinib 500 mg twice daily
-1*	Lapatinib 1000 mg once daily

-2*	Lapatinib 750 mg once daily

* The Cmax for lapatinib is higher when administered at a BID dose.
Please refer to Table 2 (background section) for specific values.

Table 5 Summary of dose holding/interruptions and dose de-escalation recommendations for lapatinib in case of lapatinib-related adverse events (graded according to NCI-CTCAE v4.0)

General Adverse Events	Action
Non-hematological, Grade 1 or 2	Continue lapatinib therapy at full dose prescribed. Apply maximum supportive care recommendations. If prolonged duration of Grade 2 adverse event (≥7 days) is affecting quality of life, decrease dose to DL -1, if symptoms persist and continue to affect quality of life for ≥ 7 more days, a second dose reduction to DL -2 will be allowed.
Non-hematological, Grade 3 or 4 (excluding cardiac and hepatobiliary events)	Apply maximum supportive care recommendations. Hold lapatinib therapy until recovery to Grade ≤ 1 (up to 14 days). For NCI-CTCAE v4.0 Grade 3 or 4 interstitial pneumonitis or Grade 4 rash manifested as toxic epidermal necrolysis (e.g. Stevens-Johnson Syndrome etc) lapatinib must be permanently discontinued If recurrence of adverse event after drug hold/ interruptions is observed, and maximum supportive care measures applied, hold drug once again until recovery to Grade ≤ 1 (up to 14 days) and restart drug at DL-1. If adverse event recurs, a second dose reduction to DL -2 will be allowed after symptoms recover to Grade ≤ 1.
Non-hematological, Grade 3 or 4 adverse events NOT resolved to Grade ≤ 1 within a maximum of 2 weeks from last planned administration	Action (discontinue or resume lapatinib therapy) in individual cases after discussions with the Sponsor. Up to 2 reductions (DL-1 and DL-2) will be considered after maximum supportive care recommendations are introduced.
Cardiac Adverse Events	
Cardiac (Severity corresponding to NYHA criteria)	Lapatinib therapy to be discontinued permanently in case of symptomatic NYHA class III and IV CHF. Lapatinib therapy to be held, continued, or resumed according to Figure 9 (please see Section 5.2.1 below) for patients with NYHA class I or II CHF.
Hepatobiliary Adverse Events	
Grade 3 AST/ALT* and Grade 2 total bilirubin elevation	Hold lapatinib for 2 weeks; restart drug at DL-1 if events resolved to ≤Grade 1. If Grade 3 events recur after dose reduction, then discontinue lapatinib.

Grade 2 AST/ALT or Grade 1 total bilirubin elevation	Hold lapatinib for 2 weeks and restart drug at full dose if events resolved to ≤ Grade 1.
Grade 4 events	Discontinue lapatinib
* retest within 3 days from the first occurrence and then weekly to determine if ALT elevation persists Note: In case of multiple short interruptions of dose due to either adverse events or drug supply or other reasons the sum of days without lapatinib treatment should not exceed 21 days in any 90 day treatment period.	

5.2.1 Management of Cardiac Events

Asymptomatic cardiac events:

Subjects who have a ≥20% decrease in LVEF relative to baseline, and the ejection fraction is below the institution's lower limit of normal, should have a repeat evaluation of ejection fraction 1-2 weeks later while still receiving investigational product.

- If the repeat ejection fraction evaluation confirms a ≥20% decrease in LVEF, and the ejection fraction is below the institution's lower limit of normal, then lapatinib should be temporarily discontinued.
- If the LVEF recovers during the next 3 weeks, after consultation and approval of the Sponsor, the subject may be restarted on lapatinib at a reduced dose. For such subjects, monitoring of LVEF will then be performed 2 weeks and 4 weeks after rechallenge, and then every 4 weeks thereafter.
- If repeat ejection fraction evaluation still shows a decrease ≥20% in LVEF relative to baseline, and the value is below the institution's lower limit of normal, then lapatinib should be stopped.

Symptomatic cardiac events:

Lapatinib should be stopped.

5.3 On Study Evaluations

5.3.1 Day 1 (10 patients who live locally at any treating site)
PK samples will be collected:

- Pre-treatment (before start of lapatinib)
- 1h, 2h, 3h, 4h, 6h, 8h post 1st dose in the morning

5.3.2 Day 14 after the start of therapy (± 5 days):

- CBC with differential and platelet count
- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- Clinic visit or phone interview by Research Nurse for review of systems.

5.3.3 At the end of Cycle 1 (after 28 days; ± 5 days):

- Physical examination
- CBC with differential and platelet count
- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- Toxicity assessment, review of patient diary, and medication reconciliation
- Pregnancy counseling including the potential risk of fetal exposure, and verification of use of male and female contraception
- CT or MRI scan with oral and IV contrast (the same studies as those done at initiation of treatment should be performed)
- PK samples (patients who had PK samples performed prior to dose 1) - before 1st dose in the morning and 1h, 2h, 3h, 4h, 6h, 8h post morning dose.

5.3.4 Days 14 of Cycle 2 and Cycle 3 (± 5 days):

- CBC with differential and platelet count
- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- Phone interview by Research Nurse for review of systems.

5.3.5 End of Course 2 Evaluation (end of Cycle 3, week 12; ± 5 days) and every 8 weeks (one course) during treatment (± 2 weeks):

- CT or MRI scan with oral and IV contrast (the same studies as those done at initiation of treatment should be performed)
- History and physical examination

- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- CBC with differential and platelet count
- Toxicity assessment, review of patient diary, and medication reconciliation
- LVEF by ECHO (end of course 2 and every 2 courses (approx 16 weeks) thereafter

Note: If clinically indicated (e.g. deterioration of performance status, increase in pain, change in relevant laboratory parameters) restaging studies (as noted above) may be done prior to the 2 month interval including repeat brain CT or MRI is warranted by clinical condition.

5.3.6 Evaluation at the End of all Other Even Cycles (d28 of each cycle, +/- 5 days)

- CBC with differential and platelet count
- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- Toxicity assessment, review of patient diary, and medication reconciliation, to be performed at clinic visit during week 4 of the first cycle of each course following course 1.

5.3.7 Dispensing of medication

After reviewing the most recent lab data and the patient diary (please see sections 5.3.3, 5.3.5, and 5.3.6 above), and determining that the patient can continue with treatment, the physician or designee will contact the pharmacy and the investigational agent will be dispensed to the patient at the clinic visit. Only enough lapatinib for one (1) cycle of therapy + one week to allow for delays in appointment scheduling or travel may be provided to the patient each cycle. Patients will be required to return any unused study drugs or empty bottles to the NIH or participating institution at each clinic visit.

5.4 Research Evaluations

5.4.1 Pharmacokinetics

PK samples will be collected, using a single 6 mL sodium heparing (green top) tube, from 10 patients who live locally at any of the treating sites and who agree to participate in the PK portion of the study. Blood will be drawn for PK analysis at the time points specified in Section 5.3.1 and 5.3.3

Determination of lapatinib concentration in all plasma samples as well as subsequent pharmacokinetic data analysis will be performed by the Clinical Pharmacology Program (CPP) (Dr. William Figg, MOB, CCR, NCI, 301-402-3622). Samples will be analyzed using a validated LC-MS-MS assay. PK parameters derived from plasma concentrations may include peak Cmax, Tmax, AUC, CL and CL/F, Vd, and t1/2

Please refer to Appendix G for collection, storage and shipment of samples.

5.4.2 Biomarker development predicting response to lapatinib in patients with metastatic melanoma harboring *ERBB4* mutations

In this study, tumor biopsies will be obtained at the Coordinating Center (Surgery Branch/NCI) only to characterize the effects of lapatinib on the biology of the tumor cells. For all patients, tumor biopsies will be optional.

5.4.2.1 *Timing of biopsy*

Biopsies will be performed at the following times:

- After consent, prior to treatment with lapatinib
- At day 14 of cycle 1 (+/-5 days)
- At day 28 of cycle 3 (+/-5 days)

5.4.2.2 *Biopsy procedure*

At least 24 hours prior to the scheduled biopsy, the research nurse will contact the laboratory of Dr. Yardena Samuels, at 301-451-2628 notifying them of the procedure.

Serial tumor biopsies will be obtained through Interventional Radiology by a percutaneous approach. Two core biopsies not less than 18-gauge in diameter and at least 1 cm in length will be obtained. Only percutaneous biopsies will be performed on patients with solid tumors. To minimize risk, serial biopsies will not be performed in deep organs of the chest and abdomen. It is estimated that there will be between 2 to 5 million cells from each biopsy. If a site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators and Interventional Radiology, an attempt at biopsy will be made. The use of imaging to facilitate biopsies will be decided by members of the Interventional Radiology team and may include ultrasound, CT scan, or MRI. Should CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies and local anesthesia will be administered only if they are considered to be of low risk to the participant as determined by the investigators and Interventional Radiology.

5.4.2.3 *Processing and Storage of the Biopsies*

The biopsies will be cut into two parts in the operating room immediately after the procedure; the larger portion of the biopsies will be put into pre-labeled cryotubes and immediately put on dry ice, and the smaller portion will be fixed with 10% neutral buffered formalin overnight. The samples will be immediately delivered to the Laboratory of Dr. Yardena Samuels in Bldg 50/Room 5140 and will be stored at a -80°C (frozen sample) or at room temperature (formalin fixed sample) until ready for analysis. Formalin fixed samples will be paraffin-embedded at Dr. Samuels laboratory.

5.4.2.4 *Studies*

Snap-frozen core-needle biopsies obtained from patients pre- and post treatment with lapatinib will be utilized to make cell lysates for the analysis of the changes in protein levels and phosphorylation status of the ERBB4 and Akt pathways:

5.4.2.4.1 *DNA Sequencing*

RAF and RAS isoforms will be sequenced by Sanger sequencing on the ABI 3730 using the forward, reverse, and sequencing primers (Appendix H) as detailed in SOP listed in Appendix I. Of the BRAF gene only exon 15 containing the V600E hotspot will be sequenced. Additionally, all exons of the CDKN2A gene (INK/p16) and TP53 will be determined on all biopsies using the sequencing primers listed in Appendix J.

5.4.2.4.2 *Genetic markers predicting response to lapatinib in melanoma harboring activating ERBB4 mutations*

Genomic DNA extracted from patients tissues and used for ERBB4 mutation analysis will be sequenced for the presence of concomitant oncogene mutations in the RAF / RAS family and the tumor suppressor genes INK4/p16 and TP53 as outlined in section 5.4.2.4.1 above. The association of response to lapatinib (PR as defined by RECIST) and presence of concomitant activating oncogene mutations or loss of tumor suppressor function will be examined in a Fisher's exact test design:

	PR, CR – response to lapatinib	PD – progression of disease
Presence of mutation		
Wild type – no concomitant mutation		

Validation of any statistically significant association will require a larger follow-up study.

5.4.2.4.3 *Correlative Protein Expression Studies*

Tissue obtained at follow-up biopsies (outlined below) will be collected, stored, and in the future analyzed by quantitative Western Blotting for phosphoprotein expression of pERBB4, pAKT, and pERK. Phospho- and total protein expressions will be compared to baseline levels, and changes will be correlated using the Fischer's exact test design for response to lapatinib. The development and translation into clinically applicable IHC testing will need to await larger studies.

Immunoprecipitation and protein blotting. Melanoma tissue from core needle biopsies will be gently washed three times in PBS and then lysed using 0.5–1.0 mL 1% NP-40 lysis buffer (1% Nonidet P-40, 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1× EDTA-free Complete Protease Inhibitor tablet (Roche), 1 μM sodium orthovanadate, 1 mM sodium fluoride and 0.1% β-mercaptoethanol) for 20 min on ice. Extracts are centrifuged for 10 min at 20,000g at 4 °C. Five hundred microliters of supernatant will be immunoprecipitated overnight using 20 μL of anti-ERBB4–conjugated agarose beads (Santa Cruz Biotechnology). The immunoprecipitates will be washed and subject to SDS-PAGE and protein blotting. Primary antibodies used in our analysis are anti-ERBB4 (Santa Cruz Biotechnology), anti–P-ERBB4(Y1162) (Abgent), anti–P-ERBB4(Y1284) (Cell Signaling), anti-PY20 (Zymed-Invitrogen), anti–P-ERK1/2 (T202/Y204), anti-ERK1/2, anti–P-AKT (S473), anti-AKT (Cell Signaling), and anti–α-tubulin (Calbiochem–EMD Biosciences).

Immunoblot quantification analysis. Scanned films from protein blot analysis of SDS-PAGE will be analyzed using ImageJ (National Institutes of Health, Bethesda, Maryland, USA). Individual bands will be quantified and plots generated to determine the intensities in each band. The data are exported to Microsoft Excel and analyzed further for the ratio of phosphoprotein to total protein.

5.5 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of lapatinib 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.

Either the Principal Investigator or an Associate Investigator should be notified of any change in the patient's therapy, if a change is indicated. All effort should be made to give only medications that are clearly indicated for a specific medical purpose. Other anti-tumor therapy will not be permitted while the patient is on study.

Patients may be admitted as necessary to manage issues related to study medication or disease process. Anti-emetic and anti-diarrheal medications will be administered as

appropriate. Guidelines for Management of Gastrointestinal Adverse Events can be found in Appendix K of this protocol.

5.6 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for up to 2 years (27 cycles), or until patients meet off treatment criteria as described in Section 5.8.

5.7 Follow-Up Evaluations

After completion of therapy, patients will be followed every three months during the first 2 years, every 4 months during the third year and as clinically indicated after that. After three years, patients who are continuing to respond will be contacted by telephone annually to determine disease status.

At each clinic visit the patient will undergo the following evaluation:

- History and physical examination
- Laboratory evaluations
- Chem 14 Panel {Albumin, alkaline phosphatase, total bilirubin, Total CO2 (bicarbonate), Urea nitrogen (BUN), calcium, chloride (Cl), creatinine, glucose, potassium(K), total protein, SGOT [AST], SGPT [ALT], sodium(Na)}
- CBC with differential and platelet count
- Imaging Studies

5.8 Off Treatment Criteria

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Pregnancy
- Patient decides to withdraw from treatment
- Patient must start on a drug with potential CYP3A4 interaction
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

5.9 Off Study Criteria

Patients will be taken off study for the following:

- The patient voluntarily withdraws
- There is significant noncompliance
- General or specific changes in the patient's condition render continued participation the patient unacceptable for further follow up in the judgment of the investigator.
- Disease Progression

Note : Prior to being taken off study, all patients will be followed for 30 days or until all adverse events have resolved to grade 2 or less following the last dose of

medication. In the event of a hepatobiliary event, patients will be followed until events have resolved to grade 1 or less following the last dose of medication.

6. DATA COLLECTION, MONITORING, AND REPORTING

6.1. Data Collection

Data will be prospectively collected and entered into the database at least once every two weeks. The NCI C3D database will be used for this study. The responsible investigator is Dr. Udo Rudloff.

Complete records must be maintained on each patient. These will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered into the NCI C3D database from which formal analyses are done. The primary source documentation will assure the following: on study information, including patient eligibility data and patient history; and off-study summary sheets, including a final assessment by the treating physician. All data will be kept secure. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.

Laboratory data collected from outside of the NIH Clinical Center will be entered into C3D manually. Training will be provided to the participating institutions by the NIH.

Data will be submitted to the CTEP/CDUS every three months via the NCI C3D database.

6.2 Data Reporting

6.2.1 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/protocolDevelopment/electronic_applications/cdus.htm).

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

6.2.2 Responsibility for Submissions

Participating institutions are responsible for submitting CDUS data and/or data forms to the Coordinating Center quarterly by January 10, April 10, July 10, October 10 to allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP (see Section 11.1.).

The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

6.3 Data and Safety Monitoring Plan

The principal investigator will review all serious adverse events and will monitor the data and toxicities to identify trends monthly. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The NCI IRB will review submitted adverse events monthly to also evaluate trends and will require a follow up plan from the principal investigator whenever a trend is identified.

A CCR Safety Monitoring Committee will monitor toxicity trends on this study on at least an annual basis and report any trends to the NCI IRB and Principal Investigator.

The principal investigator will review accrual to both the screening and enrollment phases of the trial every 12 months. If fewer participants than anticipated are enrolling in the treatment phase, the reasons for lack of enrolment will be reviewed and discussed with the Sponsor.

7. 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 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited (via AdEERS) reporting **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Lapatinib (GW572016, NSC 727989)

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 Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers for further clarification. *Frequency is provided based on 1890 patients.* Below is the CAEPR for Lapatinib (GW572016).

Version 2.4, January 6, 2010[1]

Adverse Events with Possible Relationship to Lapatinib (GW572016) (CTCAE 4.0 Term) [n= 1890]			**EXPECTED AEs FOR ADEERS REPORTING Agent Specific Adverse Event List (ASAEL)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	***Expected***
CARDIAC DISORDERS			
		Left ventricular systolic dysfunction	***Left ventricular systolic dysfunction***
GASTROINTESTINAL DISORDERS			
	Abdominal distension		***Abdominal distension***
	Abdominal pain		***Abdominal pain***
Diarrhea			***Diarrhea***
	Dyspepsia		***Dyspepsia***
	Flatulence		***Flatulence***
	Gastrointestinal disorders – Other (Mucositis/stomatitis – Select)		***Gastrointestinal disorders – Other (Mucositis/stomatitis – Select)***
Nausea			***Nausea***
	Vomiting		***Vomiting***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue***
	Flu like symptoms		***Flu like symptoms***
HEPATOBILIARY DISORDERS			
		Hepatic failure	***Hepatic failure***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased***
	Blood bilirubin increased		***Blood bilirubin increased***
		Electrocardiogram QT	***Electrocardiogram QT***

		corrected interval prolonged	***corrected interval prolonged***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia***
	Dehydration		***Dehydration***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		***Dysgeusia***
	Headache		***Headache***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Pneumonitis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Nail loss		
	Pruritus		***Pruritus***
	Rash acneiform		***Rash acneiform***
Rash maculo-papular			***Rash maculo-papular***
VASCULAR DISORDERS			
	Flushing		***Flushing***

[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.

Also reported on Lapatinib (GW572016) trials but with the relationship to Lapatinib (GW572016) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Anemia; Febrile neutropenia
CARDIAC DISORDERS - Atrial fibrillation; Restrictive cardiomyopathy
EYE DISORDERS - Blurred vision
GASTROINTESTINAL DISORDERS - Constipation; Dysphagia; Gastritis; Gastrointestinal disorders – Other (Hemorrhage, GI – Select); Gastrointestinal disorders – Other (Obstruction, GI – Select)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema limbs; Fever; Pain
INFECTIONS AND INFESTATIONS – Infections and infestations – Other (Infection – Select)
INVESTIGATIONS - Alkaline phosphatase increased; Creatinine increased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hypoalbuminemia; Hypoglycemia; Hypokalemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Myalgia
NERVOUS SYSTEM DISORDERS - Cerebrospinal fluid leakage; Depressed level of consciousness; Dizziness; Intracranial hemorrhage
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Epistaxis; Pleural effusion
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Palmar-plantar erythrodysesthesia syndrome; Skin and subcutaneous tissue disorders - Other (seborrheic dermatitis); Urticaria
VASCULAR DISORDERS - Hypotension; Thromboembolic event; Vascular disorders - Other (hypovolemia)

Note: Lapatinib (GW572016) 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.

7.2 Adverse Event Characteristics

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

- **"Expectedness": AEs can be 'Unexpected**' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1).

- **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.

7.3 Expedited Adverse Event Reporting

7.3.1 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 7.3.3).

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

7.3.2 AdEERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. AdEERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines – AdEERS Reporting Requirements for Adverse Events that occur within 30 Days[1] of the Last Dose of the

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

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected 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.

7.3.4 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting (i.e., AdEERS). The following AEs must be reported through the routine reporting mechanism (Section 7.4):

- Grade 2 or greater pain with or without hospitalization
- Events related to vascular access devices with or without hospitalization
- Grade 2 or greater lab abnormalities with or without hospitalization that do not support a reportable adverse event

7.3.5 Additional Protocol-Specific Expedited Reporting Requirements

- *Cardiac Events*
  Cardiac dysfunction will be reported as an SAE and will be defined as any signs or symptoms of deterioration in left ventricular cardiac function that are Grade 3 (NCI CTCAE) or a ≥20% decrease in LVEF relative to baseline which is below the institution's lower limit of normal. See section 5.2.1 for additional information regarding cardiac event reporting.
  Note: Refer to NCI CTCAE grading of left ventricular cardiac function.

- *Pulmonary Events*
  If a patient develops symptoms suggestive of interstitial pneumonitis, adult respiratory distress syndrome (ARDS), or non-cardiogenic pulmonary edema, lapatinib therapy should be interrupted and a thorough evaluation performed. If NCI-CTCAE v4.0 Grade 3 or 4 pneumonitis or pulmonary fibrosis is confirmed (and the relationship to lapatinib cannot be excluded), lapatinib must be permanently discontinued. All incidences of interstitial lung disease/ interstitial pneumonitis regardless of grade will be reported as serious adverse events (SAEs).

7.3.6 Expedited Reporting of Adverse Events and Deaths to the IRB

Participating sites will report expedited adverse events to CTEP and the coordinating center via AdEERs. Each institution and the coordinating center will submit the events to their IRB of record per established institutional guidelines.

At the Coordinating Center (NCI) the protocol PI will report adverse events to the NCI-IRB as per guidelines described in Section 7.6 below.

7.3.7 Expedited Reporting of Deaths on Study and Adverse Events to GSK

A copy of all AdEERS reports submitted to CTEP will also be submitted by fax by each participating institution (with a copy to the coordinating center) to the following individuals at GSK:

Yasir Naagarwala, MD
Yasir.x.nagarwala@gsk.com
Phone: 610-917-6068
Fax: 610-917-6715

Michael Arbushites
Michael.2.arbushites@gsk.com
Phone: 610-917-4039
Fax: 610-917-6715

7.4 Routine Data Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through AdEERS must also be reported in routine study data submissions.**

Data will be captured in the NCI C3D web based electronic reporting system. This study will utilize the CTCAE version 4.0 for toxicity and Adverse Event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.

7.4.1 All laboratory evaluation will be kept in the source documents. Only the following laboratory values that are drawn outside of the NIH Clinical Center will be entered in C3D:

- On study and at each the beginning of each cycle:
  - ALT, AST, Bilirubin, Creatinine,
  - ANC, hemoglobin, platelets
- At any time during the study:
  - Any grade 3 - 4 value of the above
  - Any grade 2 or greater lab abnormality that supports the diagnosis of a reportable adverse event

7.4.2 The following concomitant medications will be reported in the source documents only and will not be captured in C3D:

- PRN and one time medications
- Medications prescribed for a short period to treat an acute symptom (e.g. a course of antibiotics for a sinus infection.)

7.5 Secondary AML/MDS

AML/MDS events are now to be reported via AdEERS (in addition to your routine AR reporting mechanisms). In CTCAE v4.0, the event (s) may be reported as either:

1. Leukemia secondary to oncology chemotherapy,
2. Myelodysplastic syndrome,
3. Treatment related secondary malignancy.

For more information, please refer to:
http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/adeers.htm

7.6 NCI-IRB Adverse Event Reporting

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

At the Coordinating Center (NCI) 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.

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

The protocol PI will report to the NCI-IRB:

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

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

7.6.3 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.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with lapatinib can be found in Section 7.1.

8.1 Lapatinib (NSC 727989)

Chemical Name: N-{3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2 furyl]-4-quinazolinamine

Other Names: GW572016, Tykerb®

Molecular Formula: C29H26ClFN4O4S(C7H8O3S)2H2O

Molecular Weight: 943.48

Approximate solubility: 0.007 mg/mL in water and 0.001 mg/mL in 0.1 N HCl at 25°C.

Mode of Action: Dual inhibitor of epidermal growth factor receptor (EGFR or ErbB1) and ErbB2 tyrosine kinases.

How Supplied: Lapatinib is supplied by the NCI/DCTD as 250 mg oval, biconvex, orange film-coated tablets with one side plain and the opposite side debossed with either FG HLS or GS XJG. The tablets contain 405 mg of lapatinib ditosylate monohydrate, equivalent to 250 mg lapatinib free base per tablet. The tablets are packaged into HDPE bottles with child-resistant closures containing 90 tablets per container.

Excipients present in the tablet include: Microcrystalline cellulose, povidone, sodium starch glycolate, and magnesium stearate.

The film-coat contains: Hydroxypropyl methylcellulose, titanium dioxide, macrogel/PEG 400, Polysorbate 80, FD&C Yellow No. 6, and FCF aluminum lake.

Storage: Store intact bottles at controlled room temperature (15°C-30°C). Protect from light.

Stability: Shelf life surveillance studies of the intact bottle are on-going. Current data indicates lapatinib is stable for at least 36 months at controlled room temperature (15°C - 30°C).

Route of Administration: Oral, taken one hour before or after meals.

Method of Administration:
Whenever possible, administer whole tablets. Lapatinib tablets have not been deliberately formulated to be dispersible tablets; however, in circumstances where dosing of whole tablets is not possible, see procedure below. Tablet crushing is not recommended.

For patients unable to swallow tablets, a suspension preparation in water or Kool-Aid can be made using the following procedure:

1. Prepare Lemonade or Tropical Punch Kool-Aid as directed on package.
2. Place 2 or 4 ounces of water or Kool-Aid in a glass container, then add the required number of lapatinib tablets for dose (up to six tablets per 2 to 4 ounces) to the container.
3. Cover the container, let it stand for 5 minutes, and then stir the mixture intermittently for 15 minutes or until it is fully dispersed.
4. Stir the container for 5 seconds then administer.
5. Rinse the container with 2 ounces of water or Kool-Aid and repeat the administration process.

(The lemonade mixture appears somewhat like orange juice whereas the tropical punch mixture appears like carrot juice.)

Potential Drug Interactions:
In vitro studies with human liver microsomes indicate that lapatinib is metabolized by CYP3A4 and CYP3A5, and to a lesser extent CYP2C19 and CYP2C8. Co-administration of lapatinib with potent or moderate CYP3A4 inhibitors (including grapefruit juice) and all CYP3A4 inducers is prohibited. Assess risk/benefit before co-administering lapatinib with weak CYP3A4 inhibitors. CYP3A4 inhibitors may decrease lapatinib metabolism (increasing levels); while CYP3A4 inducers may increase lapatinib metabolism (decreasing levels).

In human subjects, lapatanib inhibited CYP3A4 and CYP2C8 at clinically relevant concentrations. Avoid co-administration of lapatinib with drugs that are substrates of CYP3A4 or CYP2C8 and have narrow therapeutic windows.

Lapatinib potentially interacts with warfarin and quinazoline derivatives to increase INR and bleeding. Collect INR/PT determinations more frequently (e.g. weekly for the first month and weekly for a minimum of 2 weeks following lapatinib discontinuation).

8.2 Availability

Lapatinib is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Lapatinib is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.3 Agent Ordering

NCI-supplied agents 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 (NIH-986) 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 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.4 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.)

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 4 weeks prior to administration of protocol therapy, unless otherwise indicated. Assessment may be performed more frequently as clinically indicated.

		Course 1			Course 2				Course 3-14		
	Pre-Study	Cy 1, d1	Cy 1, d14	Cy 1, d 28	Cy 2, d 14	Cy 2, d 28	Cy 3, d 14	Cy 3, d 28	1st Cy (even) d 28[h]	2nd Cy (odd) d 28i	Off Study[e]
Lapatinib[a]		X----------								----X	
Informed consent	X										
Erbb4 mutation screen	X										
Demographics	X										
Medical history	X										
Concurrent meds	X										
Physical examination	X			X		X		X		X	X
Performance status	X										X
CBC w/diff, plts[j]	X	X----------								----X	
Serum chemistry[b,j]	X	X----------								----X	
Urinalysis[j]	X										
B-HCG[c,j]	X										
PT/aPTT/fibrinogen or INR[j]	X										
ECHO	X							X		X[f]	
Radiologic evaluation	X			X				X		X	
Adverse event eval		X----------								----X	
Tumor measurements	X			X				X		X	
Tumor biopsy[d]	X		X					X			
PK[g]	X	X		X							

a: ***Lapatinib*****:** Dose as assigned; route/schedule.
b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
c: Serum or urine pregnancy test (women of childbearing potential).
d: Biopsy optional
e: Off-study evaluation.
f: Every other course
g On 10 patients only
h 1st cycle of each course (cycles 4, 6, 8, 10, 12, and 14)
I 2nd cycle of each course (cycles 5, 7, 9, 11 and 13)
j These baseline tests are to be conducted within 14 days prior to administration of lapatinib.

10. MEASUREMENT OF EFFECT

10.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response after completion of the first cycle, after completion of the 3rd cycle and every 8 weeks thereafter (± 2 weeks). In addition to a baseline scan, confirmatory scans should also be obtained 8 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[72] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

10.1.1 Definitions

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

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycles of therapy, 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 cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

10.1.2 Disease Parameters

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

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

Malignant lymph nodes. To be considered pathologically enlarged and

measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).[73] At baseline and in follow-up, only the short axis will be measured and followed.

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

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

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

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

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

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to

the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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

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

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

Ultrasound, Endoscopy, Laparoscopy, or Tumor markers cannot be used for disease evaluation in this study.

FDG-PET is an optional imaging modality in this study. Results from FDG-PET scans which are obtained in the routine care of patients may be captured and recorded including SUV measurements. FDG-PET cannot substitute for above imaging for disease evaluation.

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions[72]

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD):	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

10.1.4.2 Evaluation of Non-Target Lesions[72]

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
Non-CR/Non-PD:	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD):	Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump

target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

10.1.4.3 Evaluation of Best Overall Response

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

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

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.[72]

** required in this non-randomized trials with response as primary endpoint

*** On discretion of the Principal Investigator, unequivocal progression in non-target lesions may be accepted as disease progression.

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease		

10.1.5 Duration of Response

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

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

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

10.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

10.2 Antitumor Effect – Hematologic Tumors

N/A

11. REGULATORY CONSIDERATIONS

11.1 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are

presented in Appendix L.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

11.2 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, Agent-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 CFR 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: ncicteppubs@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.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

This is a phase 2 trial in patients with measurable stage IV melanoma harboring *ERBB4* mutations treated with the tyrosine kinase inhibitor lapatinib. All patients eligible for treatment will undergo formal response evaluation after 1 and 3 cycles of therapy and every 2 cycles after that.

Screening
The prevalence of metastatic cutaneous melanoma patients harboring no more than two *ERBB4* mutations is 17%. If this were to apply to the screening population and if 180 patients were screened, there is an 88.9% probability of identifying at least 25 patients with an *ERBB4* mutation.

Treatment
The primary objective of this trial is to determine if lapatinib monotherapy is able to produce adequate numbers of clinical responses in patients with metastatic melanoma harboring *ERBB4* mutations. If so, this regimen will be considered for use in subsequent studies, to determine its role in this disease.

The study will be conducted using a phase 2 MinMax design.[74] The objective of the trial will be to determine whether, this novel agent is able to be associated with a response rate (PR + CR) that can rule out 10% (p0=0.10) in favor of an improved response rate of 30% (p1=0.30). Using alpha=0.10 (probability of accepting a poor agent) and beta=0.10 (probability of rejecting a good agent), initially 16 evaluable patients will be enrolled in the study. If 0 to 1 of 16 patients respond (PR +CR), then no further patients will be enrolled. If 2 or more of the first 16 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 25 evaluable patients have been enrolled. If 2 to 4 of the 25 have a clinical response, then this will be considered inadequate for further investigation of this regimen. If 5 or more of 25 respond, then this will warrant further investigation in a subsequent trial. Under the null hypothesis (10% response rate), the probability of early termination is 51%. If a DLT is seen in the first three patients, dose de-escalation will occur as described in section 3.3.

A variety of secondary evaluations will be performed, corresponding to the secondary

objectives listed. In all cases, the evaluations will be done with exploratory intent, and will be performed using non-parametric tests and descriptive statistics on however many samples are available. If the study terminates after only 16 patients then no further patients will be enrolled to obtain biologic endpoint data.

12.2 Sample Size/Accrual Rate

The accrual ceiling will be set at 180 patients to be screened for the presence of ERBB4 mutations to allow for treatment of up to 28 patients, which would include up to 3 inevaluable patients and based on the calculation described above (Section 12.1). It is expected that 1-3 patients per month will be treated on this trial. Allowing for treatment with this agent over a two year period with follow up, it is expected that the trial will be completed in approximately 3-5 years.

12.3 Stratification Factors

N/A

12.4 Analysis of Secondary Endpoints

Progression-free survival will be calculated using the Kaplan-Meier method.

Toxicity data will be obtained, and if there are 5 or more patients who experience Grade 3 or greater toxicity attributable to the agent, then comparisons between this cohort and patients who tolerated the same course of lapatinib without toxicity across grades of toxicity with respect to outcome (response) and clinicopathological parameters (age, sex, type of visceral involvement) may be done using a Kruskal-Wallis test. Otherwise, toxicities will be tabulated and described.

The association of response to lapatinib (PR as defined by RECIST) and presence of concomitant activating oncogene mutations or loss of tumor suppressor function will be examined in a Fischer's exact test.

Comparisons between the pERBB4 : total ERBB4 and phospho Akt : total Akt ratio will be made by subtracting pre-treatment from posttreatment levels and determining the statistical significance of the difference using a Fischer's exact test. Similarly, pErk and pSTAT levels will be evaluated for a change from baseline levels. These measures will all be evaluated with exploratory intent, and findings will be reported without formal adjustment for multiple comparisons, but in the context of a study with multiple exploratory analyses performed.

12.5 Reporting and Exclusions

12.5.1 Evaluation of toxicity – All patients will be evaluable for toxicity from the time of their first treatment with lapatinib.

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

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific. All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

13. HUMAN SUBJECTS PROTECTION

13.1 Rationale for Subjects Protection

- Selection based on gender, ethnicity, and race: Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information which suggests that differences in drug metabolism or disease response would be expected in any one patient group. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of patients exposed to potentially toxic treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other. Prior toxicologic and clinical evaluations have not specifically assessed effects on juvenile animals. Therefore, this study will not include patients < 18 years of age. This is a Phase II trial designed to define the response rate, characterize the side effect profile, and assess several biological endpoints.

13.2 Evaluation of Benefits and Risks/Discomforts

Potential benefits to subjects expected from the trial: As a result of participating in this trial, patients will receive evaluation and treatment of their tumor. This protocol may or may not be helpful for a specific patient, but the results may help the investigators learn about the administration of lapatinib on a continuing basis. The

potential for this research treatment to offer control of the disease is unknown; however, based on information to date, the possibility of an effect on the progression of disease exists. Benefit cannot be promised nor can the chance of benefit be accurately predicted.

13.3 Consent and Assent Processes and Documents

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits and potential alternative therapies will be carefully explained to the patient or the patient's surrogate, and a signed informed consent document will be obtained by the PI, AI or clinical staff fellow. Moreover, any experimental invasive procedure will require a separate consent form.

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009; 59:225-49.
2. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Ding S, Byrd DR, Cascinelli N, Cochran AJ, Coit DG, Eggermont AM, Johnson T, Kirkwood JM, Leong SP, McMasters KM, Mihm MC, Jr., Morton DL, Ross MI, Sondak VK. Multivariate Analysis of Prognostic Factors Among 2,313 Patients With Stage III Melanoma: Comparison of Nodal Micrometastases Versus Macrometastases. J Clin Oncol. 2010; 28:2452-9.
3. Desmond RA, Soong SJ. Epidemiology of malignant melanoma. Surg Clin North Am 2003; 83:1-29.
4. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004; 351:998-1012.
5. FDA. Databases on the FDA website. wwwaccessdatafdagov/scripts/cder/onctools/druglistcfm 2009.
6. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, Paradise C, Kunkel L, Rosenberg SA. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999; 17:2105-16.
7. Bellett RE, Mastrangelo MJ, Laucius JF, Bodurtha AJ. Randomized prospective trial of DTIC (NSC-45388) alone versus BCNU (NSC-409962) plus vincristine (NSC-67574) in the treatment of metastatic malignant melanoma. Cancer Treat Rep 1976; 60:595-600.
8. Middleton MR, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, Seiter S, Gore M, Aamdal S, Cebon J, Coates A, Dreno B, Henz M, Schadendorf D, Kapp A, Weiss J, Fraass U, Statkevich P, Muller M, Thatcher N. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol 2000; 18:158-66.
9. Weber JS, O'Day S, Urba W, Powderly J, Nichol G, Yellin M, Snively J, Hersh E. Phase I/II study of ipilimumab for patients with metastatic melanoma. J Clin Oncol 2008; 26:5950-6.
10. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, Sherry RM, Topalian SL, Kammula US, Royal RE, Restifo NP, Haworth LR, Levy C, Mavroukakis SA, Nichol G, Yellin MJ, Rosenberg SA. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol

2005; 23:6043-53.
11. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002; 298:850-4.
12. Dudley ME, Rosenberg SA. Adoptive cell transfer therapy. Semin Oncol 2007; 34:524-31.
13. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 2005; 23:2346-57.
14. Hocker TL, Singh MK, Tsao H. Melanoma genetics and therapeutic approaches in the 21st century: moving from the benchside to the bedside. J Invest Dermatol 2008; 128:2575-95.
15. Sawyers C. Targeted cancer therapy. Nature 2004; 432:294-7.
16. Flaherty K PI, Sosman J, Kim K, Ribas A, McArthur G, Lee RJ, Grippo JF, Nolop K, Chapman P. Phase I study of PLX4032: Proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. J Clin Oncol 2009; 27: 15 suppl.
17. Carvajal RD CP, Wolchok JD, Cane L, Teitcher JB, Lutzky J, Pavlick AC, Bastian BC, Antonescu CR, Schwartz GK. A phase II study of imatinib mesylate (IM) for patients with advanced melanoma harboring somatic alterations of *KIT.* J Clin Oncol 2009; 27: 15 suppl.
18. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature 2002; 417:949-54.
19. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004; 116:855-67.
20. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004; 64:7099-109.
21. Haluska F, Pemberton T, Ibrahim N, Kalinsky K. The RTK/RAS/BRAF/PI3K pathways in melanoma: biology, small molecule inhibitors, and potential applications. Semin Oncol 2007; 34:546-54.
22. Eisen T, Ahmad T, Flaherty KT, Gore M, Kaye S, Marais R, Gibbens I, Hackett S, James M, Schuchter LM, Nathanson KL, Xia C, Simantov R, Schwartz B, Poulin-Costello M, O'Dwyer

PJ, Ratain MJ. Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. Br J Cancer 2006; 95:581-6.
23. Agarwala SS, Keilholz, U., Hogg, D., et al. Randomized phase III study of paclitaxel plus carboplatin with or without sorafenib as second-line treatment in patients with advanced melanoma. ASCO Meeting Abstract 2007; 25:Abstract 8510.
24. Lorusso PM, Adjei AA, Varterasian M, Gadgeel S, Reid J, Mitchell DY, Hanson L, DeLuca P, Bruzek L, Piens J, Asbury P, Van Becelaere K, Herrera R, Sebolt-Leopold J, Meyer MB. Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. J Clin Oncol 2005; 23:5281-93.
25. LoRusso PM, Krishnamurthi SS, Rinehart JJ, Nabell LM, Malburg L, Chapman PB, DePrimo SE, Bentivegna S, Wilner KD, Tan W, Ricart AD. Phase I pharmacokinetic and pharmacodynamic study of the oral MAPK/ERK kinase inhibitor PD-0325901 in patients with advanced cancers. Clin Cancer Res; 16:1924-37.
26. Board RE, Ellison G, Orr MC, Kemsley KR, McWalter G, Blockley LY, Dearden SP, Morris C, Ranson M, Cantarini MV, Dive C, Hughes A. Detection of BRAF mutations in the tumour and serum of patients enrolled in the AZD6244 (ARRY-142886) advanced melanoma phase II study. Br J Cancer 2009; 101:1724-30.
27. Stahl JM, Sharma A, Cheung M, Zimmerman M, Cheng JQ, Bosenberg MW, Kester M, Sandirasegarane L, Robertson GP. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res 2004; 64:7002-10.
28. Tolcher AW, Yap, T.A., Fearen, I., Taylor, A., Carpenter, C., Brunetto, A.T., Beeram, M., Papadopoulos, K., Yan, L., de Bono, J. A phase I study of MK-2206, an oral potent allosteric Akt inhibitor (Akti), in patients (pts) with advanced solid tumor (ST). J Clin Oncol 2009; 27:suppl; abstr 3503.
29. Vukelja S, Richards, D., Campos, L.T., Bedell, C., Hagenstad, C., Hyman, W., Letzer, J., Gardner, L., Sportelli, P., Nemunaitis, J. Randomized phase II study of perifosine in combination with capecitabine versus capecitabine alone in patients with second- or third-line metastatic colon cancer. J Clin Oncol 2009; 27:suppl; abstr 4081.
30. Rao RD, Allred, J.B., Windschitl, H.E., Maples, W.J., McWilliams, R.R., Creagan, E.T., Kaur, J.S., Kottchade, L.A., Gornet, M.K., Pockaj, B.A., Markovic, S.N. . N0377: Results of NCCTG phase II trial of the mTOR inhibitor RAD-001 in metastatic melanoma. J Clin Oncol 2007; 15:Suppl; abstr 8530.
31. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006; 24:4340-6.
32. Wyman K, Atkins MB, Prieto V, Eton O, McDermott DF, Hubbard F, Byrnes C, Sanders K, Sosman JA. Multicenter Phase II trial of high-dose imatinib mesylate in metastatic melanoma: significant toxicity with no clinical efficacy. Cancer 2006; 106:2005-11.
33. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, Bremer R, Gillette S, Kong J, Haass NK, Sproesser K, Li L, Smalley KS, Fong D, Zhu YL, Marimuthu A, Nguyen H, Lam B, Liu J, Cheung I, Rice J, Suzuki Y, Luu C, Settachatgul C, Shellooe R, Cantwell J, Kim SH, Schlessinger J, Zhang KY, West BL, Powell B, Habets G, Zhang C, Ibrahim PN, Hirth P, Artis DR, Herlyn M, Bollag G. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci U S A 2008; 105:3041-6.
34. Rusnak DW, Lackey K, Affleck K, Wood ER, Alligood KJ, Rhodes N, Keith BR, Murray DM, Knight WB, Mullin RJ, Gilmer TM. The effects of the novel, reversible epidermal

growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. Mol Cancer Ther 2001; 1:85-94.
35. Spector NL, Xia W, Burris H, 3rd, Hurwitz H, Dees EC, Dowlati A, O'Neil B, Overmoyer B, Marcom PK, Blackwell KL, Smith DA, Koch KM, Stead A, Mangum S, Ellis MJ, Liu L, Man AK, Bremer TM, Harris J, Bacus S. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. J Clin Oncol 2005; 23:2502-12.
36. Lackey KE. Lessons from the drug discovery of lapatinib, a dual ErbB1/2 tyrosine kinase inhibitor. Curr Top Med Chem 2006; 6:435-60.
37. Qiu C, Tarrant MK, Choi SH, Sathyamurthy A, Bose R, Banjade S, Pal A, Bornmann WG, Lemmon MA, Cole PA, Leahy DJ. Mechanism of activation and inhibition of the HER4/ErbB4 kinase. Structure 2008; 16:460-7.
38. Kumar A, Petri ET, Halmos B, Boggon TJ. Structure and clinical relevance of the epidermal growth factor receptor in human cancer. J Clin Oncol 2008; 26:1742-51.
39. Rudloff U, Samuels Y. A growing family: Adding mutated Erbb4 as a novel cancer target. Cell Cycle; 9.
40. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304:1497-500.
41. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004; 101:13306-11.
42. Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Shibata T, Sakiyama T, Yoshida T, Tamura T. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol 2005; 23:6829-37.
43. Bence AK, Anderson EB, Halepota MA, Doukas MA, DeSimone PA, Davis GA, Smith DA, Koch KM, Stead AG, Mangum S, Bowen CJ, Spector NL, Hsieh S, Adams VR. Phase I pharmacokinetic studies evaluating single and multiple doses of oral GW572016, a dual EGFR-ErbB2 inhibitor, in healthy subjects. Invest New Drugs 2005; 23:39-49.
44. Burris HA, 3rd, Hurwitz HI, Dees EC, Dowlati A, Blackwell KL, O'Neil B, Marcom PK, Ellis MJ, Overmoyer B, Jones SF, Harris JL, Smith DA, Koch KM, Stead A, Mangum S, Spector NL. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. J Clin Oncol 2005; 23:5305-13.
45. Burris HA, 3rd, Taylor CW, Jones SF, Koch KM, Versola MJ, Arya N, Fleming RA, Smith DA, Pandite L, Spector N, Wilding G. A phase I and pharmacokinetic study of oral lapatinib administered once or twice daily in patients with solid malignancies. Clin Cancer Res 2009; 15:6702-8.
46. LoRusso PM, Jones SF, Koch KM, Arya N, Fleming RA, Loftiss J, Pandite L, Gadgeel S, Weber BL, Burris HA, 3rd. Phase I and pharmacokinetic study of lapatinib and docetaxel in patients with advanced cancer. J Clin Oncol 2008; 26:3051-6.
47. Chu QS, Schwartz G, de Bono J, Smith DA, Koch KM, Versola MJ, Pandite L, Arya N,

Curtright J, Fleming RA, Ho PT, Rowinsky EK. Phase I and pharmacokinetic study of lapatinib in combination with capecitabine in patients with advanced solid malignancies. J Clin Oncol 2007; 25:3753-8.
48. Chu QS, Cianfrocca ME, Goldstein LJ, Gale M, Murray N, Loftiss J, Arya N, Koch KM, Pandite L, Fleming RA, Paul E, Rowinsky EK. A phase I and pharmacokinetic study of lapatinib in combination with letrozole in patients with advanced cancer. Clin Cancer Res 2008; 14:4484-90.
49. Medina PJ, Goodin S. Lapatinib: a dual inhibitor of human epidermal growth factor receptor tyrosine kinases. Clin Ther 2008; 30:1426-47.
50. GlaxoSmithKline. Lapatinib [package insert]. GlaxoSmithKline, Research Triangle Park, NC 2007.
51. FDA ApfAnN-. Medical reviews [FDA Web site]. http://wwwfdagov/cder/foi/nda/022059s000TOChtm 2008.
52. Koch KM, Reddy NJ, Cohen RB, Lewis NL, Whitehead B, Mackay K, Stead A, Beelen AP, Lewis LD. Effects of food on the relative bioavailability of lapatinib in cancer patients. J Clin Oncol 2009; 27:1191-6.
53. Gomez HL, Doval DC, Chavez MA, Ang PC, Aziz Z, Nag S, Ng C, Franco SX, Chow LW, Arbushites MC, Casey MA, Berger MS, Stein SH, Sledge GW. Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer. J Clin Oncol 2008; 26:2999-3005.
54. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006; 355:2733-43.
55. Cameron D, Casey M, Press M, Lindquist D, Pienkowski T, Romieu CG, Chan S, Jagiello-Gruszfeld A, Kaufman B, Crown J, Chan A, Campone M, Viens P, Davidson N, Gorbounova V, Raats JI, Skarlos D, Newstat B, Roychowdhury D, Paoletti P, Oliva C, Rubin S, Stein S, Geyer CE. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 2008; 112:533-43.
56. Burstein HJ, Storniolo AM, Franco S, Forster J, Stein S, Rubin S, Salazar VM, Blackwell KL. A phase II study of lapatinib monotherapy in chemotherapy-refractory HER2-positive and HER2-negative advanced or metastatic breast cancer. Ann Oncol 2008; 19:1068-74.
57. Blackwell KL, Pegram MD, Tan-Chiu E, Schwartzberg LS, Arbushites MC, Maltzman JD, Forster JK, Rubin SD, Stein SH, Burstein HJ. Single-agent lapatinib for HER2-overexpressing advanced or metastatic breast cancer that progressed on first- or second-line trastuzumab-containing regimens. Ann Oncol 2009; 20:1026-31.
58. Iwata H, Toi, M., Fujiwara, Y. et al. Phase II clinical study of lapatinib (GW572016) in patients with advanced or metastatic breast cancer. Abstract; Breast Cancer Symposium; December 14-17; San Antonio, Texas 2006; Abstr. 1091.
59. Di Leo A, Gomez HL, Aziz Z, Zvirbule Z, Bines J, Arbushites MC, Guerrera SF, Koehler M, Oliva C, Stein SH, Williams LS, Dering J, Finn RS, Press MF. Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer. J Clin Oncol 2008; 26:5544-52.
60. Crown JP, Burris HA, 3rd, Boyle F, Jones S, Koehler M, Newstat BO, Parikh R, Oliva C,

Preston A, Byrne J, Chan S. Pooled analysis of diarrhea events in patients with cancer treated with lapatinib. Breast Cancer Res Treat 2008; 112:317-25.
61. Lacouture ME, Laabs SM, Koehler M, Sweetman RW, Preston AJ, Di Leo A, Gomez HL, Salazar VM, Byrne JA, Koch KM, Blackwell KL. Analysis of dermatologic events in patients with cancer treated with lapatinib. Breast Cancer Res Treat 2009; 114:485-93.
62. Perez EA, Koehler M, Byrne J, Preston AJ, Rappold E, Ewer MS. Cardiac safety of lapatinib: pooled analysis of 3689 patients enrolled in clinical trials. Mayo Clin Proc 2008; 83:679-86.
63. Prickett TD, Agrawal NS, Wei X, Yates KE, Lin JC, Wunderlich JR, Cronin JC, Cruz P, Rosenberg SA, Samuels Y. Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in ERBB4. Nat Genet 2009; 41:1127-32.
64. Gross ME, Shazer RL, Agus DB. Targeting the HER-kinase axis in cancer. Semin Oncol 2004; 31:9-20.
65. Harari PM, Allen GW, Bonner JA. Biology of interactions: antiepidermal growth factor receptor agents. J Clin Oncol 2007; 25:4057-65.
66. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sanchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ, Taron M. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009; 361:958-67.
67. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009; 361:947-57.
68. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Ruschoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353:1659-72.
69. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007; 25:1960-6.
70. LoPiccolo J, Blumenthal GM, Bernstein WB, Dennis PA. Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. Drug Resist Updat 2008; 11:32-50.
71. Prickett TD, Agrawal, N.S., Wei, X., Yates, K.E., Lin, J.C., Wunderlich, J., Cronin, J.C., Cruz, P., NISC Comparative Sequencing Program, Rosenberg, S.A., Samuels, Y. Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in ERBB4. Nat Genet 2009; in press.
72. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45:228-47.
73. Schwartz LH, Bogaerts J, Ford R, Shankar L, Therasse P, Gwyther S, Eisenhauer EA.

Evaluation of lymph nodes with RECIST 1.1. Eur J Cancer 2009; 45:261-7.
74. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials 1989; 10:1-10.

APPENDIX A: CLINICAL TRIALS OF LAPATINIB IN BREAST CANCER

Clinical Trials of Lapatinib in Patients with Advanced or Metastatic Breast Cancer[a]

Authors	Patient Population	No.	Treatment Regimens	Response
Burstein et al.[73] Phase II International OL	Cohort A: HER2-positive MBC refractory to an anthracycline, a taxane, capacitabine, and trastuzumab Cohort B: HER2-negative MBC refractory to an anthracycline, a taxane, and capecitabine	140 89	Lapatinib 1,500 mg QD	Response RR: 4.3%; clinical benefit: 5.7%; median TTP: 9.1 wk; median PFS: 9.1 wk; median survival: 29.4 wk No objective responses; median TTP: 7.6 wk; median PFS: 7.6 wk; median survival: 18.6 wk
Blackwell et al.[74] Phase II MC, OL	HER2-positive MBC refractory to trastuzumab	78	Lapatinib 1,500mg QD	PR: 7.7%; clinical benefit: 21.8%
Iwata et al.[75] Phase II MC, OL	HER2-positive MBC refractory to an anthracycline, a taxane, capecitabine, and trastuzumab	45	Lapatinib 1,500mg QD	Clinical benefit: 33.3%
Gomez et al.[76] Phase II MC, R, OL	First-line treatment of HER2-positive advanced breast cancer or MBC	138	Lapatinib 1,500mg QD (n=69) or Lapatinib 500mg BID (n=69)	RR: 24%; clinical benefit: 31%; PFS at 6 mo: 43%; no difference in clinical activity between doses
Geyer et al.[77] and Cameron et al.[78] Phase III MC, R, OL	HER2-positive locally advanced breast cancer or MBC that had progressed with regimens including an anthracycline, a taxane, and trastuzumab	399	Lapatinib 1,250mg QD + capacitabine 1,000 mg/m2 PO BID on days 1-14 of a 21-day cycle or Capacitabine 1,250mg/m2 PO BID on days 1-14 of a 21-day cycle	Median TTP: 6.2 mo (combination) vs 4.3 mo (capacitabine alone) (HR=0.57; 95% CI, 0.43-0.77; p<0.001); improved PFS with combination vs capacitabine alone (HR=0.55; 95% CI, 0.4-0.74; p<0.001)
DiLeo et al.[79] Phase III MC, R, DB, PC	First-line therapy of HER2-negative or unknown advanced breast cancer or MBC	580	Lapatinib 1,500mg QD + paclitaxel 175mg/m2 IV q3wk or Placebo + paclitaxel 175mg/m2 IV q3wk	RR: 35.1% vs 25.3%, combination vs paclitaxel monotherapy, respectively (p=0.008); clinical benefit: 40.5% vs 31.9% (p=0.025); no difference in TTP or OS

[a]from Medina PJ, Goodin S. Clin Ther 2008

APPENDIX B: ERBB4 PRIMERS

GENE	Exon	Primer Name	Non M13 tagged Forward Primer	Primer Name	Non M13 tagged Reverse Primer
ERBB4	1b	ERBB4-X1b-F	GGGGATATGCCATTTGGAC	ERBB4-X1b-R	CGGAGTGCCAGAAGGAAC
ERBB4	2	ERBB4-X2-F	AGAACTGGGATAGGCTTGTGG	ERBB4-X2-R	TTCCAGGTATCAGCACACAGG
ERBB4	3b	ERBB4-X3b-F	GGCAACTGTTTGTGTCTTTTCA	ERBB4-X3b-R	AAGCATATTTGCCATTTTGGA
ERBB4	4b	ERBB4-X4b-F	TTCATCAACAAGCAGTTTGACA	ERBB4-X4b-R	TCGCCACATAGGGTAGAACA
ERBB4	5	ERBB4-X5-F	AAATCCTCATAAAGGAGCAGGAG	ERBB4-X5-R	CCAAAGCAAATCAACCACAAG
ERBB4	6	ERBB4-X6-F	TGAATTGAGTCAAAGACAGGGTG	ERBB4-X6-R	GGAATGACTTTGAGGAGGGC
ERBB4	7b	ERBB4-X7b-F	TGAAAGTAATATTTGCTGTGTTGC	ERBB4-X7b-R	TTCTTTTGATTTCAAATAATGACCT
ERBB4	8b	ERBB4-X8b-F	TGTTTTGAGCTTGTTTGCTGA	ERBB4-X8b-R	AAACCTTGTTATATAGGCCCAGTTC
ERBB4	9b	ERBB4-X9b-F	TTGGCCAAAAATAAGTTTCTCAA	ERBB4-X9b-R	CACTTTGTAAAATAACTTGCACAAAAA
ERBB4	10b	ERBB4-X10b-F	AAATTTGGCTACATCTCTTCTTGA	ERBB4-X10b-R	AAATTATATTGTTCATAGCGCAACA
ERBB4	11	ERBB4-X11-F	CCTTTCTCACTTCCCAACTTTC	ERBB4-X11-R	TACCTCACACCATCATCGGAG
ERBB4	12b	ERBB4-X12b-F	TTTTCTCACTTCCCCCTCCT	ERBB4-X12b-R	TCCAAAGAAGAATGGGAAAAA
ERBB4	13b	ERBB4-X13b-F	TCCCCTTGATTTTGGTGTTT	ERBB4-X13b-R	ATGAGGTGAAGGCAACCCTA
ERBB4	14	ERBB4-X14-F	TGATGCTCCTGGCACATAGAG	ERBB4-X14-R	CCCATGGCATCCTGTAAGTAG
ERBB4	15	ERBB4-X15-F	TCTTAGAGGAAGATTTGCCACC	ERBB4-X15-R	CATTTCAGAGATGGTACCAGGG
ERBB4	16	ERBB4-X16-F	GCTTCCCATGTTCTTCCTCC	ERBB4-X16-R	AAGTAAGAAAGTTGGCTTGAGAAGG
ERBB4	17	ERBB4-X17-F	TGTGGATAATGTCTTGTACAACTGC	ERBB4-X17-R	TTCACAAGCTTTGTTTAACGGAC
ERBB4	18b	ERBB4-X18b-F	TTCTTCTTTCCGCTTTGCAG	ERBB4-X18b-R	TCCATTGGCTATTATTTTCTAAACA
ERBB4	19b	ERBB4-X19b-F	TGTAACAGGTGCTAAATAAACATTTG	ERBB4-X19b-R	TGATTGCCTGGGTGTCTGTA
ERBB4	20b	ERBB4-X20b-F	TTGAGTTGAAATCATGGTATTGC	ERBB4-X20b-R	TTCCATAGAAATTGACAGGCACT
ERBB4	21b	ERBB4-X21b-F	GGGAAAACCTGGGCATTAAC	ERBB4-X21b-R	TCAAGCAAGATTGCTCTCAAAA
ERBB4	22	ERBB4-X22-F	AGGCCAGCCCAAAGACTC	ERBB4-X22-R	TAACTGCTTTAGGAAATTAGGCTTATC
ERBB4	23b	ERBB4-X23b-F	TTGGTGTTTGGATTGACCTG	ERBB4-X23b-R	TGATGGTGATAACATTATTTTGCAG
ERBB4	24	ERBB4-X24-F	GAGTCGTTTCTTTCACTAGCTTGC	ERBB4-X24-R	TGTTTGTGGTCCTTTCCACAG
ERBB4	25b	ERBB4-X25b-F	TGTGTCTGATGGGCAATCTT	ERBB4-X25b-R	TTATTTTGAAATGTTAGTGCTTATGAA
ERBB4	26b	ERBB4-X26b-F	CCATCATTCCATTTTTCTTTCC	ERBB4-X26b-R	AAGCAAAGACCGAAAATCCT
ERBB4	27b	ERBB4-X27b-F	ACAACGCCTTCTCTCCACAT	ERBB4-X27b-R	AATGGCGATCGTTTCTGAAT
ERBB4	28_1b	ERBB4-X28_1b-F	TTTTCCAGAAACTAGAGGTTAGCTG	ERBB4-X28_1b-R	GGTAGTCTGGGTGCTGAAGG
ERBB4	28_2b	ERBB4-X28_2b-F	AGGCCGAGGATGAGTATGTG	ERBB4-X28_2b-R	GGAAATTGGAGCAGGTGTGT

APPENDIX C: 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 D: List of drugs that may have potential CYP3A4 interactions

List of drugs that may have potential CYP3A4 interactions (source: ctep.cancer.gov) [Updated on May 1, 2007]

When drugs classified as 'substrates' are co-administered with lapatinib there is the potential for higher concentrations of the 'substrate'. When lapatinib is co-administered with compounds classified as 'inhibitors', increased plasma concentrations of lapatinib is the potential outcome. The co-administration of 'inducers' would potentially lower plasma lapatinib concentrations. Only major substrates and effective inducers are listed.

CYP3A4 Substrates

Albuterol	Dihydroergotamine	Isradipine	Quinidine
Alfentanil	Diltiazem	Itraconazole	Rabeprazole
Alprazolam	Disopyramide	Ketamine	Ranolazine
Amiodarone	Docetaxel	Ketoconazole	Repaglinide
Amlodipine	Doxepin	Lansoprazole	Rifabutin
Amprenavir	Doxorubicin	Letrozole	Ritonavir
Aprepitant	Doxycycline	Levonorgestrel	Salmeterol
Aripiprazole	Efavirenz	Lidocaine	Saquinavir
Atazanavir	Eletriptan	Losartan	Sibutramine
Atorvastatin	Enalapril	Lovastatin	Sildenafil
Benzphetamine	Eplerenone	Medroxyprogesterone	Simvastatin
Bisoprolol	Ergoloid mesylates	Mefloquine	Sirolimus
Bortezomib	Ergonovine	Mestranol	Spiramycin
Bosentan	Ergotamine	Methadone	Sufentanil
Bromazepam	Erythromycin	Methylergonovine	Sunitinib
Bromocriptine	Escitalopram	Methysergide	Tacrolimus
Budesonide	Estradiol	Miconazole	Tamoxifen
Buprenorphine	Estrogens, conj., synthetic	Midazolam	Tamsulosin
Buspirone	Estrogens, conj., equine	Miglustat	Telithromycin
Busulfan	Estrogens, conj., esterified	Mirtazapine	Teniposide
Carbamazepine	Estrone	Modafinil	Tetracycline
Cerivastatin	Estropipate	Montelukast	Theophylline
Chlordiazepoxide	Ethinyl estradiol	Moricizine	Tiagabine
Chloroquine	Ethosuximide	Nateglinide	Ticlopidine
Chlorpheniramine	Etoposide	Nefazodone	Tipranavir
Cilostazol	Exemestane	Nelfinavir	Tolterodine
Cisapride	Felbamate	Nevirapine	Toremifene
Citalopram	Felodipine	Nicardipine	Trazodone
Clarithromycin	Fentanyl	Nifedipine	Triazolam
Clobazam	Flurazepam	Nimodipine	Trimethoprim
Clonazepam	Flutamide	Nisoldipine	Trimipramine
Clorazepate	Fluticasone	Norethindrone	Troleandomycin
Cocaine	Fosamprenavir	Norgestrel	Vardenafil
Colchicine	Gefitinib	Ondansetron	Venlafaxine
Conivaptan	Haloperidol	Paclitaxel	Verapamil
Cyclophosphamide	Ifosfamide	Pergolide	Vinblastine
Cyclosporine	Imatinib	Phencyclidine	Vincristine
Dantrolene	Indinavir	Pimozide	Vinorelbine
Dapsone	Irinotecan	Pipotiazine	Zolpidem
Dasatinib (1)	Isosorbide	Primaquine	Zonisamide
Delavirdine	Isosorbide dinitrate	Progesterone	Zopiclone
Diazepam	Isosorbide mononitrate	Quetiapine	

CYP3A4 Inhibitors

Acetominophen	Diclofenac	Lomustine	Primaquine
Acetazolamide	Dihydroergotamine	Losartan	Progesterone
Amiodarone	Diltiazem	Lovastatin	Propofol
Amlodipine	Disulfiram	Mefloquine	Propoxyphene
Amprenavir	Docetaxel	Mestranol	Quinidine
Anastrozole	Doxorubicin	Methadone	Quinine
Aprepitant	Doxycycline	Methimazole	Quinupristin
Atazanavir	Drospirenone	Methoxsalen	Rabeprazole
Atorvastatin	Efavirenz	Methylprednisolone	Ranolazine
Azelastine	Enoxacin	Metronidazole	Risperidone
Azithromycin	Entacapone	Miconazole	Ritonavir
Betamethasone	Ergotamine	Midazolam	Saquinavir
Bortezomib	Erythromycin	Mifepristone	Selegiline
Bromocriptine	Ethinyl estradiol	Mirtazapine	Sertraline
Caffeine	Etoposide	Mitoxantrone	Sildenafil
Cerivastatin	Felodipine	Modafinil	Sirolimus
Chloramphenicol	Fentanyl	Nefazodone	Sulconazole
Chlorzoxazone	Fluconazole	Nelfinavir	Tacrolimus
Cimetidine	Fluoxetine	Nevirapine	Tamoxifen
Ciprofloxacin	Fluvastatin	Nicardipine	Telithromycin
Cisapride	Fluvoxamine	Nifedipine	Teniposide
Clarithromycin	Fosamprenavir	Nisoldipine	Testosterone
Clemastine	Glyburide	Nizatidine	Tetracycline
Clofazimine	Grapefruit juice (2)	Norfloxacin	Ticlopidine
Clotrimazole	Haloperidol	Olanzapine	Tranylcypromine
Clozapine	Hydralazine	Omeprazole	Trazodone
Cocaine	Ifosfamide	Orphenadrine	Troleandomycin
Conivaptan	Imatinib	Oxybutynin	Valproic acid
Cyclophosphamide	Indinavir	Paroxetine	Venlafaxine
Cyclosporine	Irbesartan	Pentamidine	Verapamil
Danazol	Isoniazid	Pergolide	Vinblastine
Dasatinib (1)	Isradipine	Phencyclidine	Vincristine
Delavirdine	Itraconazole	Pilocarpine	Vinorelbine
Desipramine	Ketoconazole	Pimozide	Voriconazole
Dexmedetomidine	Lansoprazole	Pravastatin	Zafirlukast
Diazepam	Lidocaine	Prednisolone	Ziprasidone

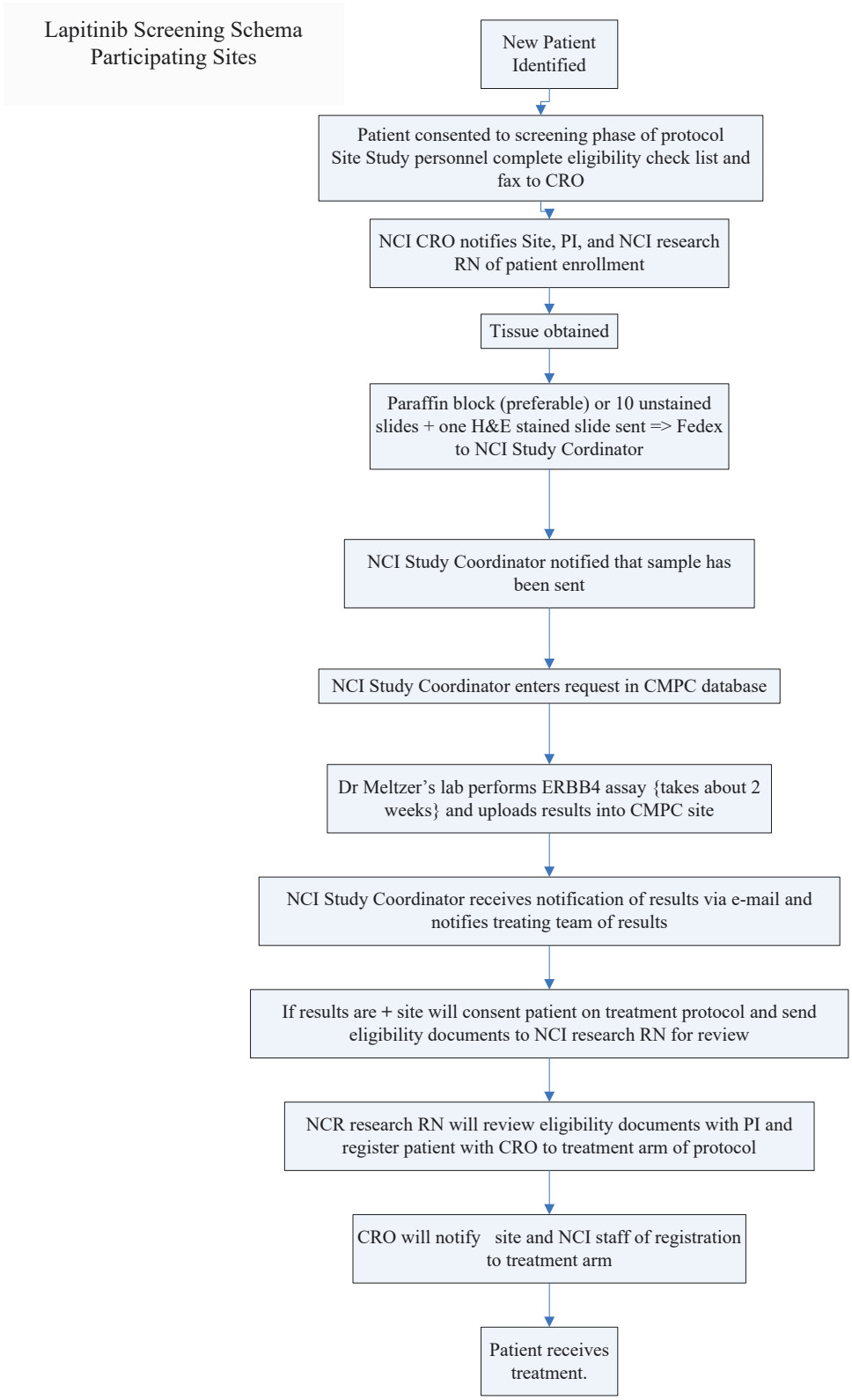
CYP3A4 Inducers

Aminoglutethimide	Nevirapine	Phenytoin	Rifapentine
Carbamazepine	Oxcarbazepine	Primidone	St. John's wort (3)
Fosphenytoin	Pentobarbital	Rifabutin	
Nafcillin	Phenobarbital	Rifampin	

Note: Adapted from Cytochrome P450 Enzymes: Substrates, Inhibitors, and Inducers. In: Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 15TH ed. Hudson, OH; LexiComp Inc. 2007: 1899-1912.
Only major substrates and effective inducers are listed.
Additional information for drug interactions with cytochrome P450 isoenzymes can be found at http://medicine.iupui.edu/flockhart/.

(1) Investigator's Brochure: Dasatinib (BMS 354825). Bristol-Myers Squibb. October 2006.
(2) Malhotra *et al*. (2001). Clin Pharmacol Ther. 69:14-23.
(3) Mathijssen *et al*. (2002). J Natl Cancer Inst. 94:1247-1249.
Frye *et al*. (2004). Clin Pharmacol Ther. 76:323-329.

APPENDIX E: LAPATINIB SCREENING SCHEMA



APPENDIX F: Patient Medication Diary

Appendix 7 Patient Diary

Name:

take lapatinib - one pill twice daily - 1 hour before or after breakfast and dinner

Mon	Tue	Wed	Thu	Fri	Sat	Sun
12 . morning [ ] evening [ ]	13 . morning [ ] evening [ ]	14 . morning [ ] evening [ ]	15 . morning [ ] evening [ ]	16 . morning [ ] evening [ ]	17 . morning [ ] evening [ ]	18 . morning [ ] evening [ ]
19 . morning [ ] evening [ ]	20 . morning [ ] evening [ ]	21 . morning [ ] evening [ ]	22 . morning [ ] evening [ ]	23 morning [ ] evening [ ] Lab Work RN will call	24 . morning [ ] evening [ ]	25 . morning [ ] evening [ ]
26 . morning [ ] evening [ ]	27 . morning [ ] evening [ ]	28 . morning [ ] evening [ ]	29 . morning [ ] evening [ ]	30 . morning [ ] evening [ ]	1 . morning [ ] evening [ ]	2 . morning [ ] evening [ ]
3 . morning [ ] evening [ ]	4 . morning [ ] evening [ ]	5 . morning [ ] evening [ ]	6 . morning [ ] evening [ ]	7 morning [ ] evening [ ] Lab Work Clinic visit	8 . morning [ ] evening [ ]	9 . morning [ ] evening [ ]

List all symptoms whether or not you think they are related to the lapatinib	Dates	Treated at Home	Treated in doctor's office or Clinic	Hospitalized	Comments
Diarrhea					
Nausea					
Vomiting					
Other GI symptoms (describe)					
Skin rash (describe)					
Other symptoms					

APPENDIX G: Laboratory Methodology for Pharmacokinetics Studies

Laboratory Methodology

Pharmacokinetic Studies

Pharmacokinetics of lapatinib

Venous blood samples should be collected in a 6-ml sodium heparin (green top) tube at the following time points:

- Pre-treatment (before start of lapatinib)
- 1h, 2h, 3h, 4h, 6h, 8h post 1st dose in the morning (up to pre evening dose)
- Day 28 (± 5 days) – before 1st dose in the morning and 1h, 2h, 3h, 4h, 6h, 8h post morning dose (up to pre-evening dose).

For samples collected at the Surgery Branch, NCI, immediately place specimens on wet ice and refrigerate. The date and **exact** time of each blood draw should be recorded on the sample tube and the PK sheet. Please page 102-11964 (Gareth Peters or alternate tech) for immediate pick-up. (Contact the Clinical Pharmacology Program (CPP) processing group in 10/5A09 at 301-594-6131 or 301-402-3622 with any questions).

Instructions for Specimen Processing and Shipping (collaborating institutions)

Specimen Processing

1. Draw specimen in 6ml Na Heparin tube (green top)
2. Place blood tubes on wet ice immediately after draw - keep cold (4C) until aliquots are placed in the freezer.
3. Process samples within one hour of draw whenever possible.
4. Spin at 2400rpm (1250g) for 5min at 4C
5. After centrifugation, split each Na heparin tube sample into 2 aliquots and store aliquots in cryovials (ex. Nunc 375418).
6. Store at -80 C
7. Provide the date and time of draw both on the frozen tube and PK sheet. Provide patient's assigned study code and protocol number on PK sheet. Put patient study code and protocol number on tubes to link them to PK sheet.
8. Do not allow samples to thaw once frozen.

Specimen Shipping

1. Place samples in a cardboard sample box with an absorbent strip (ex. Saf-T-Pak #STP-152 250ml absorbent)
2. Place sample box and PK sheet in a ziplock bag (ex. Lab Guard # SBL2X810B)
3. Package specimen bag with dry ice inside an insulated shipper (ex. Saf-T-Pak #STP-300 Category A shipping overpak).

Note: Any shipping packaging approved for a Category A substance is acceptable; dry ice is considered a hazardous material and thus must be handled by appropriately trained individuals.

4. Ship via FedEx Priority Overnight to:

William Figg

National Cancer Institute
9000 Rockville Pike
Bldg 10 Rm 5A01
Bethesda, MD 20892
Tel: 301-402-3622

5. Ship only on Mon-Wed. Do not ship the day before a federal holiday.
6. Send PK sheets with shipment.
7. If shipping samples for more than one pt, separate out patient specimens in separate ziplock bags.
8. Notify me via email (comptok@mail.nih.gov) or fax (301 402-8606) on the date of shipment. Provide contact information for the person in charge of shipping at that institute as well as the number of samples shipped.

Sample Data Collection

All samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in the Patient Sample Data Management System (PSDMS) utilized by the CPP. This is a secure program, with access to the PSDM System limited to defined CPP personnel, who are issued individual user accounts. Installation of PSDMS is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All CPP personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

PSDMS creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without PSDMS access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the PSDM System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

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.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the PSDMS. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

APPENDIX H: Primers used for PCR amplification and sequencing of RAS and Raf isoforms

Table S6. Primers used for PCR amplification and sequencing of RAS and Raf isoforms

Gene and Exon Name	Forward Primer	Reverse Primer	Sequencing Primer
ARAF-1	TGAGCAGGATCTCTTGGACTG	GTAAAACGACGGCCAGTCAGCTGATGTTCCCATCCTTC	GTAAAACGACGGCCAGT
ARAF-2	GTCATGGAAGCGAAATGGC	GTAAAACGACGGCCAGTCAGAAGGAATCAAATGACTGAGG	GTAAAACGACGGCCAGT
ARAF-3	GTAAAACGACGGCCAGTTATCCCTCTGAGCCTGTTTCC	AGCAGGGAAGTTTGAGACCTG	GTAAAACGACGGCCAGT
ARAF-4	GTAAAACGACGGCCAGTTACCCAACCTCCCACTCATTC	CACGGGTCAGCTGTCTGTAAG	GTAAAACGACGGCCAGT
ARAF-5	AGTACCAACCGCCAACAGTG	GTAAAACGACGGCCAGTGAGAAATGAGGTGACTTGCCC	GTAAAACGACGGCCAGT
ARAF-6	CAAATGCATGTTTATGGCTGG	GTAAAACGACGGCCAGTCATCAGTGCTGAAACTCTGGC	GTAAAACGACGGCCAGT
ARAF-7	GTAAAACGACGGCCAGTCAACGTCCATATGGTCAGCAC	CTGGTGACTTGGAATGTGGG	GTAAAACGACGGCCAGT
ARAF-8	CCAGAGTTTCAGCACTGATGG	GTAAAACGACGGCCAGTCCAGATGGGTGGCATCTAAG	GTAAAACGACGGCCAGT
ARAF-9	GTAAAACGACGGCCAGTAGAAAGTGGTATGCTCGAGGG	ATGTCCAGGAAGCACTCCAAG	GTAAAACGACGGCCAGT
ARAF-10	GTAAAACGACGGCCAGTAGAACAGTGCCACTCCTGATG	TCTCGGTGGATGATGTTCTTG	GTAAAACGACGGCCAGT
ARAF-11	GGATTTGCCATCATCACACAG	GTAAAACGACGGCCAGTCCCTTGTAATTCCCAGAACCC	GTAAAACGACGGCCAGT
ARAF-12	GTAAAACGACGGCCAGTCAAGGTTGTGGATAGTTTGCG	GTGGGACATGAGGAGTCCAG	GTAAAACGACGGCCAGT
ARAF-13	GTGTGGGTGGCTCTGAGTTG	GTAAAACGACGGCCAGTTGGCATTTATCAGTGCAAAGG	GTAAAACGACGGCCAGT
ARAF-14	GTAAAACGACGGCCAGTAGCAGAGAATCCTCCCAAGTC	TCACATCTGCCTCCATCTCAG	GTAAAACGACGGCCAGT
ARAF15[#]	GTGTGTGTTTCACCATGAGGC	GTAAAACGACGGCCAGTGGCAGAGACGAACATTGATTG	GTAAAACGACGGCCAGT
BRAF-1	GTAAAACGACGGCCAGTAGCTCTCCGCCTCCCTTC	AAGGTGGCTGAGGGCATC	GTAAAACGACGGCCAGT
BRAF-2	GTAAAACGACGGCCAGTGAGGAACACTGGCAGTTACTGTG	TCTCTTCCCAAATCTATTCCTAATCC	GTAAAACGACGGCCAGT
BRAF-3	TGGTGTTGTATCTGACCTAGTAACCC	GTAAAACGACGGCCAGTCCTCATATGGCCTACAGTATTTCTTC	GTAAAACGACGGCCAGT
BRAF-4	GTAAAACGACGGCCAGTCCTCCCTCACTGTTACTAGCCC	TTACTCATCCATATTTCACATTCCC	GTAAAACGACGGCCAGT
BRAF-5	GTAAAACGACGGCCAGTGTGTAGTCCATCTATTATCTTGAACCC	GGGAGAGAAATACTGTCCATTCC	GTAAAACGACGGCCAGT
BRAF-6	GTTGTTTCTGAGAATGGAATTTGA	GTAAAACGACGGCCAGTTCTGAGTGGTATGATAAGTTATTTGGG	GTAAAACGACGGCCAGT
BRAF-7	GTAAAACGACGGCCAGTCAGCTTTGGCAGTATTGGATT	TCATCAGAGAGAAACCAGAAGC	GTAAAACGACGGCCAGT
BRAF-8	GTAAAACGACGGCCAGTGGTTTACATTGGCAAGTGCTTC	GTTACTTGAAAGAGGCAAGTATAAAGG	GTAAAACGACGGCCAGT
BRAF-9	GTAAAACGACGGCCAGTTTTGGCCCATCCTTTCCA	GCAGTGCCGTAGAAATATGCTT	GTAAAACGACGGCCAGT
BRAF-10	CTTCCTGTATCCCTCTCAGGC	GTAAAACGACGGCCAGTCGAACAGTGAATATTTCCTTTGATG	GTAAAACGACGGCCAGT
BRAF-11	GTAAAACGACGGCCAGTCCATGGAACAAACAAGGTTGG	AATAGTTGCTACCACTGGGAACC	GTAAAACGACGGCCAGT
BRAF-12	CAATTGCCAGTCTCTTCTAAATGTATC	GTAAAACGACGGCCAGTTTAGCATCCTTATGTTCCTGGAC	GTAAAACGACGGCCAGT
BRAF-13	GTAAAACGACGGCCAGTCATGGATAAATAGGCTTGACTGG	CCTATACATGCATGCACAATCC	GTAAAACGACGGCCAGT
BRAF-14	TCATCCTAACACATTTCAAGCC	GTAAAACGACGGCCAGTTTTGTGAATACTGGGAACTATGAAA	GTAAAACGACGGCCAGT
BRAF-15	GAATTCATCTGGCAATGATGGT	GTAAAACGACGGCCAGTTTCACGCTTACCCAGGAGTTA	GTAAAACGACGGCCAGT
BRAF-16	CCATCTATGATGTGGCATTGG	GTAAAACGACGGCCAGTCTCAACCCTCATGAAGCCATC	GTAAAACGACGGCCAGT
BRAF-17	GCTTTCTTGTAAAGTGTGATGGG	GTAAAACGACGGCCAGTCCACACAAGTGTTCTTTGGTTC	GTAAAACGACGGCCAGT
RAF1-1	GTAAAACGACGGCCAGTCTGGTCCCATTTTCCTCATC	ATAGGGGGTGTGGAAGGAAC	GTAAAACGACGGCCAGT
RAF1-2	GTAAAACGACGGCCAGTTATTTCTGTGCCCACCTTTCC	AGGTATTGGTCATCAGGCCC	GTAAAACGACGGCCAGT
RAF1-3	GTAAAACGACGGCCAGTGGCCTTGAGCAAATTACCTTC	TTGCCTTACTGTAAACAACAGCA	GTAAAACGACGGCCAGT
RAF1-4	GTAAAACGACGGCCAGTGGAGGCCAAGAAATGAAGTTG	ATGAATGCCACCAAACCTAGC	GTAAAACGACGGCCAGT
RAF1-5	GTAAAACGACGGCCAGTAAAGCAAGGCATGCTGATTG	CCACAGAAGCAGCAAGGG	GTAAAACGACGGCCAGT
RAF1-6	GTAAAACGACGGCCAGTGGTTGTACAGGTAGAGTTTGCCC	TCCTTGATCAGATTTGAAACCC	GTAAAACGACGGCCAGT
RAF1-7	GTAAAACGACGGCCAGTTGAAAAATCAGCCTTGGAGA	TTGGCAGGAGGTACTGTTGTC	GTAAAACGACGGCCAGT
RAF1-8	GGATGCAATTCGAAGTCACAG	GTAAAACGACGGCCAGTACTGTCTGATGCAAGTGTGCC	GTAAAACGACGGCCAGT
RAF1-9	AACAGATGACATGGGTTGATCC	GTAAAACGACGGCCAGTCTCAGTCCTCTCCTCCTCCTG	GTAAAACGACGGCCAGT
RAF1-10	GTAAAACGACGGCCAGTTGGAAATTTGCCGTATCTGTG	GCGGCACAGTCCACTAACTC	GTAAAACGACGGCCAGT
RAF1-11	GTAAAACGACGGCCAGTGACAGCACAGAACCCACTGTC	GGCTTGTGCAAAGATATCACAG	GTAAAACGACGGCCAGT
RAF1-12	GTAAAACGACGGCCAGTCCCTGTGTGTAACACTCCTTGG	TTCCTGTCCTCTGCCTCTTTC	GTAAAACGACGGCCAGT
RAF1-13	GCTGTGACAGAGGTAAGGTGG	GTAAAACGACGGCCAGTAATCTACAATTGCCCTGAGGC	GTAAAACGACGGCCAGT
RAF1-14	GTAAAACGACGGCCAGTCTAATGAAAGGGACAGCCTGG	AGCCTCTTCATTGCTTTGGG	GTAAAACGACGGCCAGT
RAF1-15	CAGGTAAGTCTGTGCTGGTGC	GTAAAACGACGGCCAGTCCATCTTGTAGAGGACCTGGG	GTAAAACGACGGCCAGT
RAF1-16	CCCAGGTCCTCTACAAGATGG	GTAAAACGACGGCCAGTAAACATGTGTTCTGCCTCTGG	GTAAAACGACGGCCAGT
HRAS-1	TGGGTCATTAAGAGCAAGTGG	GTAAAACGACGGCCAGTTAGAGGAAGCAGGAGACAGG	GTAAAACGACGGCCAGT
HRAS-2	GTAAAACGACGGCCAGTGAGAGGCTGGCTGTGTGAAC	GACATGCGCAGAGAGGACAG	GTAAAACGACGGCCAGT
HRAS-3	TTCCTGTGTGTGTTTGCCATC	GTAAAACGACGGCCAGTAGTGAGTGCTGCTCCCTGG	GTAAAACGACGGCCAGT
HRAS-4	GCCTCCTGTCCTCTCTGC	GTAAAACGACGGCCAGTAGCTCTCCCCAAGGACCTC	GTAAAACGACGGCCAGT
KRAS-1	GTAAAACGACGGCCAGTAAAGCCCACTGTAAGCTGGTG	AGAGAAGCAGGCCTAAGGTTG	GTAAAACGACGGCCAGT
KRAS-2	GTAAAACGACGGCCAGTCTGTTCCGGATGGAGATATGG	TTTCAATGCTCCTCTTCCCTC	GTAAAACGACGGCCAGT
KRAS-3	GTAAAACGACGGCCAGTAAGCTTGGCATAGTCCCTGAC	ACTCGAGTCACAGCACAGGC	GTAAAACGACGGCCAGT
KRAS-4	ATTTCCACATTCGAGGCTGAG	GTAAAACGACGGCCAGTGCAATGCTAACATGGGAAGG	GTAAAACGACGGCCAGT
NRAS-1	GTAAAACGACGGCCAGTCAAATGGAAGGTCACACTAGG	GAACTCAACACTGAGTTTGCAATAG	GTAAAACGACGGCCAGT
NRAS-2	GTAAAACGACGGCCAGTAATAGCATTGCATTCCCTGTG	CTCTGGTTCCAAGTCATTCCC	GTAAAACGACGGCCAGT
NRAS-3	GTAAAACGACGGCCAGTAAGCAGTCTGCCCTCCTCAG	CAAGAGACAGAGGCTGCAGTG	GTAAAACGACGGCCAGT
NRAS-4	GCCTGTTCTTGTGATTCAATAGG	GTAAAACGACGGCCAGTTGTGCAGAAGAGGATAGGCAG	GTAAAACGACGGCCAGT

[#]The primer pair did not meet our quality criteria that ≥90% of bases in the target region have a Phred quality score of at least 20 in three quarters of the tumor samples analyzed.

APPENDIX I: SOP - DNA PCR and Sequencing Assays Using the ABI 3730

Standard Operating Procedure

Clinical Molecular Profiling Core
Genetics Branch, CCR, NCI
50 South Drive, MSC 8000
Building 50, Room 5140
Bethesda MD 20892-8000
CLIA # 21D1092530

DNA PCR and Sequencing Assays Using the ABI 3730

Overview: This standard operating procedure describes the technical details of Sanger sequencing of an extant DNA sample produced using the laboratory's CLIA certified protocol. The procedure for DNA extraction from anatomic pathology specimens for clinical testing is described elsewhere. Details regarding the Sanger assay sensitivity, specificity, and validation for particular mutations being tested for are also provided elsewhere.

Workflow:

1) DNA analytes are pipetted into a 96-well plate in a pre-PCR environment where the DNA is safe from contamination.
2) Perform polymerase chain reaction (PCR) using the selected CLIA-certified test primers.
3) Purify amplified DNA in preparation for sequencing reaction.
4) Run the sequencing reactions in order to produce uniform peak heights, optimize the signal balance, and produce long reads.
5) Perform second purification to remove any unincorporated dye terminators.
6) Set up the Applied Biosystems 3730 DNA Analyzer, and set the program for the selected plates.
7) Run the 3730 DNA Analyzer and analyze the DNA sequence data using the software program Variant Reporter™.

Reagents & Supplies:

A. Components of Applied Biosystems Mix:

1. 2X AmpliTaq Gold Master Mix (Applied Biosystems Cat. # 4327059)
2. Ultra Pure Glycerol (Invitrogen Cat. # 15514-011)

B. PCR:

1. 5-10 ng/μl DNA
2. Selected 0.6 μm Primers (diluted from 100 μM Primers)

3. Water, ultra pure, unfiltered, deionized by reverse osmosis, endotoxin tested, one liter (Quality Biological, Inc. Cat #: 118-162-131)
4. 2X AmpliTaq Gold Master Mix (Applied Biosystems Cat. # 4327059)
5. Ultra Pure Glycerol (Invitrogen Cat. # 15514-011)

C. PCR Purification:
   1. Exo-Sap It (USB Cat. # 78205)

D. Sequencing Reaction:
   1. Ultra pure deionized H_2O (Quality Biological, Inc., Cat. # 118-162-131)
   2. BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Cat. # 4337456)
   3. Hi-Di™ Formamide (Applied Biosystems Cat. # 4311320)
   4. BigDye® Terminator v1.1, v3.1 5X Sequencing Buffer (Applied Biosystems Cat. # 4336699)

E. Purification:
   1. BigDye XTerminator Purification Kit (Applied Biosystems, Cat. # 4376484)

F. General Supplies:
   1. MicroAmp® 96-well Reaction Plate (Applied Biosystems Cat. # N801-0531)
   2. MicroAmp® Optical Adhesive Film (Applied Biosystems Cat. # 4311971)

Equipment:

A. ABI 3730 (Applied Biosystems Cat. # 4373905/4364619/4364620)
B. SORVALL Legend RT Plus Centrifuge (Thermo Scientific Cat. # 75004373)
C. Thermo Savant DNA 120 SpeedVac®
D. Alpha Innotech FluorChem® 8900
E. PTC-225 DNA Engine Tetrad Thermal Cycler (BioRad {formerly MJ Research} Cat. # PTC-0225)
F. Illumina High-Speed Microplate Shaker (VWR International, catalog # 13500-890).

Assay Procedure:

For DNA extraction and primer design, please see the relevant SOPs.

I. PCR Reaction:

Protocol:

1) Add three uL of PCR Primer set (0.6 uM) into each well. Dry down primer plates.
2) Add the PCR Master Mix. Mix with corresponding DNA to primer plates
3) Follow recipes in Excel Spreadsheet (see file on CMPC shared folder named "3730_Meltzer_Template_96well(current).xls")

n=	16	n=1	Stock	Unit	Final		
(uL)	160	10	2	X	1	X	Amplitaq Gold PCR Master Mix, Lot # _____
(uL)	0	0	0.6	uM	0.24	uM	RSA primers, diluted 50%, robot disp 3ul -dried down,
(uL)	16	1	10	ng/ul	10	ng	Template DNA (5ng/ul) - robot dispensed 2ul
(uL)	51.2	3.2	50	%	8	%	Glycerol (Invitgoren only), Made ___/___/___ by _____
(uL)	92.8	5.8					H2O
(uL)	320	20					

4) Select "VSEQR" on the DNA Engine Tetrad / PTC-225 Peltier Thermal Cycler.

PCR Cycling	vseqr	
	96	5 min
40 cycles	94	30 sec
	60	45 sec
Thermal cycler used	72	45 sec
_____	72	10 min
	10	Hold

5) Run a 1-2% Agarose Gel (See 1-2% Agarose Gel Electrophoresis protocol)

II. PCR Purification Protocol:

Exo-Sap-IT Protocol:

1) Add 2-4 uL of ExoSap-It enzyme reagent to each PCR well.
2) Perform the reaction using a thermocycler under the following conditions:

37	30 min
80	15 min
10	hold

III. ABI Sequencing Reaction:

(See Applied Biosystems Variant seqr protocol).

1) In 96 well reaction trays, add 2 µL each of the PCR amplicons into the appropriate wells.
2) Spin down the plates to get rid of bubbles.
3) Add 8 uL of sequencing mix as noted below.

n=	100	n=1	0	Unit	Final		
(uL)	160	1.6	2.5	X	0.4	X	Big Dye 3.1, Lot # _____
(uL)	120	1.2	5	X	0.6	X	Big Dye Sequencing Buffer
(uL)	32	0.32	10	uM	320	nM	M13 for **OR** M13 Rev primer,
	0	2					ExoSAP- treated PCR product
(uL)	488	4.88					H2O

800 10

4) Cover and place in the DNA Engine Tetrad / PTC-225 Peltier Thermal Cycler and select the program "m13seq" using the following conditions:

Cycle Sequencing		m13seq
	96	1 min
25 cycles	96	10 sec
	50	5 sec
	60	4 min
	10	Hold

Label a new set of 96 well plates before proceeding to the Qiagen DyeEx 96 Kit Protocol.

IV. Sequencing Reaction Purification:

Protocol:
The sequencing reactions can be purified using the BigDye XTerminator Kit from Applied Biosystems.

1. Add 45µL of SAM solution directly to each sequencing reaction well (heat SAM solution to 37°C if precipitates are present).
2. Vortex well to resuspend BigDye Xterminator Beads and add 10µL to each reaction well.
3. Seal plate with foil and vortex at 1800rpm for 30 minutes on an Illumina High-Speed Microplate Shaker.
4. Centrifuge the plate at 1000 xg for 2 minutes.
5. The plate is now ready to be loaded onto the 3730 DNA Analyzer. Check that the instrument protocol is set to BDx-FastSeq50_Pop7_v3_Z to ensure proper capillary height during injection.

V. Quick Guide to Applied Biosystems 3730 DNA Analyzer:

1. Turn on the 3730 DNA Analyzer instrument.
2. Log into the Applied Biosystems computer that is connected to the 3730 DNA Analyzer hardware using the following information:
   Username: Administrator
   Password: MeltzerLab
3. Run Applied Biosystems Software (Foundations 3730 Data Collection program). Go to Plate Manager and enter File name. The file name should include: **[Gene]_[gene section or region]_[sample ID]_[PI] _[plate ID]_[F or R]_[date run]** (denoting F for forward primer and R for reverse primer).
4. At Run Scheduler add the files in the order in which you would like the plates to be run. The bottom plate will be run first.
5. Clean out all trays labeled *Buffer*, *Water*, and *Waste* by rinsing with water. Refill deionized water up to the black lines in the *Water* and *Waste* trays. Add 1X 3730 Applied

Biosystems buffer (after diluting original 10X concentration) into tray labeled *Buffer*. Place tray back into the 3730 DNA Analyzer.

6. If bubbles are present in the capillary tubes, you MUST run Bubble Wizard to remove bubbles. Sample plates must be loaded in the *In* tray to run this program. If a bubble is present and the Bubble Wizard is not run, the capillary tubes can be damaged.
7. Change Sequencing Buffer in small glass jar next to the DNA polymer and fill the jar to the red line.
8. Close doors, and turn off lights (if turned on) by pushing the button with the light bulb on the left side of the 3730 DNA Analyzer instrument. The light should be green on the outside of the 3730 DNA Analyzer. If the computer is ready to run, a green *Play* button should be highlighted on the computer in the program under Run Scheduler. Click to run.
9. Discard plates after they have been run.

VI. DNA Analysis Using Variant Reporter:

1. Open and import all files.
2. Import the associated reference sequence file.
3. Click '*Analyze button*.'
4. Click on Variant Review tab for visual chromatogram analysis of all samples.
5. Save sequencing project in the following format: PI_CMPC study ID_Gene name_date of sequencing.

VII. Resulting Reads:

Ultimately, the scoring is the judgment of the technician and director reviewing the results. There are standard quality control measures, but we only read sequence that is well above the level of these cutoffs - in other words, we are reading in the best part of reads. Generally, we strive for bidirectional reads. On those occasions when the complexity of the sequence on one end is just so low that getting one of the directions to work is really difficult, but the other strand can be sequenced quite well, a judgment call will be made by the CLIA director as to whether to accept the current results, to rerun the sample, or report out with a caveat that the results are suspect.

A failed read needs to be repeated if that exon has been shown in other cancer patients to contain a possible mutation. Questionable reads should also be repeated if there is a known hotspot. Instead of "questionable" we will use the term "lower quality" or LQ.

We will rely upon the software to correctly call all negative bases but a few spot checks (~6) will be performed by the technician as a QC measure. However, the CLIA laboratory director or his designate will visually review any mutations prior to reporting the results to the clinician. In general, we will not do validation of mutations; that can be kicked back to the investigator. For some projects, validation might be important, but due to limited resources we will discuss on a case by case basis, first internally, and then with the investigator, if appropriate. In general, good quality bidirectional sequence is definitive. Result reporting nomenclature will be guided by the Update on Nomenclature for Human Gene Mutations, Ad Hoc Committee on Mutation Nomenclatures[1].

VIII. Quality Control:

PCR quality control:

QC data tracking will be documented via the PCR Amplicon Quality Control Data Tracking form.

1. **Positive controls:** No external positive control (PC) is necessary for this assay, rather the amplification of the patient DNA is proof that the PCR reagents and method are functioning correctly.
2. **Negative controls:** No template controls (NTC) should be run for each primer set used in the assay for each run. These are to control against contamination of the reagents or reaction environment from foreign DNA or amplicons. A positive NTC rules out against the use of that primer reaction in the assay and must subsequently be repeated after making use of fresh reagents and cleaning the work area with 10% bleach.
3. **Gel electrophoresis:** All NTCs and a random sampling of the patient amplicons will be run on an agarose gel to show that there is no contamination and to confirm that the reagents and PCR method is working as expected.

Acceptable limits: no band for NTCs and visible bands of the expected size for patient amplicons. Results will not be reported out if controls do not pass QC.

DNA sequencing quality control:

The *Quality Number* generated by Variant Reporter is a numerical scale (ranging from 0-99) that is used in order to compare the quality of the sample sequence with the GenBank reference sequence. Generally, the Quality Number for the sample sequence should be 5 or above in order to make an accurate comparison with the GenBank reference sequence. However, sometimes a case may occur where the forward or reverse sequence may be of low quality, while its corresponding forward or reverse sequence may be above a quality number of 5 and satisfactory. In this case, use the higher quality sequence to compare with the reference sequence. Every so often, a region of the higher quality sequence may be unreadable. When this occurs, look at the same region of the sequence in the reverse direction. If that region is clear, then review that particular region and continue analysis, but if that region is also unreadable then that sample sequence should be marked as *Failure*. Ultimately, however, the Quality Number is only a guide and the final determination of sequence quality is the technician's. The test read should also be compared to the normal sequence generated during the validation stage of the test.

IX. Troubleshooting and Corrective Actions:

1. If plate is loaded incorrectly into the ABI 3700, look for two problems: (1) Is the white lid snapped tightly onto the plate holder (2) Are the capillary needles still straight? Solution (1) is to readjust the lid for the plate holder. Solution (2) is to shut off the ABI 3700 and seek technical support to readjust capillary needles.

2. If error has occurred overnight then fix the error and either run same plate again or rerun Dye-Ex clean up using the saved plate from the sequencing reaction.

X. References

1. Cotton, RGH. *Update on Nomenclature for Human Gene Mutations*. Human Mutation (1996), 8:197-202.
2. Patrick C. Ma, Maria S. Tretiakova, Alexander C. MacKinnon, Nithya Ramnath, Candace Johnson, Sascha Dietrich, Tanguy Seiwert, James G. Christensen, Ramasamy Jagadeeswaran, Thomas Krausz, Everett E. Vokes, Aliya N. Husain, Ravi Salgia, *Expression and mutational analysis of MET in human solid cancers*. Genes, Chromosomes and Cancer (2008), 47 (12); 1025-1037.
3. *Fluorescent DNA Sequencing on the ABI 3730/3730xl***.** Mayo Clinic: Department of Laboratory Medicine and Pathology Molecular Genetics Laboratory, Rochester, MN 55905.
4. *Fluorescence-based Dideoxy Sequencing - ABI Prism BigDye Terminator Cycle Sequencing Kit.* Division of Laboratory Medicine, Clinical Center, NIH, Bethesda 20892

DNA PCR and Sequencing Assays Using the ABI 3730

SOP Revision Tracking Sheet

	Prepared by: Robert Chang, BS, and Marbin Pineda, MS, Daniel C Edelman, PhD			
	Date	**By**	**Signature**	**Comments**
Adopted		Jonathan K. Killian, MD, PhD		Original SOP for CLIA
□Reviewed □Revised				
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Discontinued:				

APPENDIX J: Primers for CDKN2A and TP53 genes

Coding Exon #	Genomic position	Forward primer - Relative position †	Forward primer sequence§	Reverse primer - Relative position ‡	Reverse primer sequence§
1	chr9:21964676-21964826	-114	M13-AGCACCGGAGGAAGAAAGAG	66	AGCGCTACCTGATTCCAATTC
2	chr9:21960900-21961207	-73	M13-GGGCTCTACACAAGCTTCCTTTC	67	CCGGGCTGAACTTTCTGTG
3	chr9:21958227-21958241	-294	TCTTCTTGCAACCCTGCG	59	M13-GGCAGTTGTGGCCCTGTAG
1	chr9:21984137-21984453	-101	M13-ATCTTGGAGGTCCGGGTG	69	CCTGGGCTAGAGACGAATTATC
2	chr9:21961001-21961207	-72	M13-GGCTCTACACAAGCTTCCTTTC	60	CTATGCGGGCATGGTTACTG
1	chr17:7520563-7520637	-74	M13-AGGGTTGGAAGTGTCTCATGC	139	AGCCCAACCCTTGTCCTTAC
2	chr17:7520424-7520446	-179	M13-CAGTCAGATCCTAGCGTCGAG	120	AAATCATCCATTGCTTGGGAC
3	chr17:7520036-7520315	-117	M13-ACGTTCTGGTAAGGACAAGGG	69	GAGGAATCCCAAAGTTCCAAAC
4	chr17:7519095-7519279	-154	M13-AAGCTCCTGAGGTGTAGACGC	69	GGGCCAGACCTAAGAGCAATC
5	chr17:7518901-7519014	-98	M13-CTGCTCAGATAGCGATGGTG	169	AGGCCCTTAGCCTCTGTAAGC
6	chr17:7518223-7518333	-286	M13-CATCCTGGCTAACGGTGAAAC	70	AGAAATCGGTAAGAGGTGGGC
7	chr17:7517743-7517880	-92	M13-GTTGGGAGTAGATGGAGCCTG	81	TTGGGCAGTGCTAGGAAAGAG
8	chr17:7517577-7517651	-107	M13-GGAGCACTAAGCGAGGTAAGC	110	TTGTCTTTGAGGCATCACTGC
9	chr17:7514651-7514758	-222	M13-ATTGCACCATTGCACTCCC	81	AGCTGCCTTTGACCATGAAG
10	chr17:7513651-7513733	-124	M13-CCATCTTGATTTGAATTCCCG	76	ATTGCAAGCAAGGGTTCAAAG

APPENDIX K: Guidelines for Management of Gastrointestinal Adverse Events

If GI adverse events are not appropriately managed, they may be associated with the development of dehydration. Management of gastrointestinal adverse events is discussed in detail below.

Nausea, vomiting, or both

In subjects who have emesis and are unable to retain lapatinib, every attempt should be made to obtain control of nausea and vomiting. A dose may be repeated if tablets can be visually found after the vomiting episode.

Diarrhea

These broad general management principles are recommended to proactively try and avoid more serious complications by active management of diarrhea syndrome. Guidelines such as these should never replace sound clinical judgment. Experience thus far suggests that when lapatinib is used as monotherapy, uncomplicated Grade 1 or 2 diarrhea is most prevalent. These general management principles do not address comprehensive management of more serious or protracted diarrhea syndromes.

Common clinical sense with the onset of uncomplicated Grade 1-2 diarrhea: stop all lactose containing products: drink 8-10 large glasses of clear liquids a day; eat frequent small meals; for Grade 2 diarrhea hold cytotoxic chemotherapy, and consider a dose reduction of lapatinib (discuss with Sponsor); administer standard doses of loperamide: initial dose 4 mg followed by 2 mg every 4 hours or after every unformed stool. It is suggested to continue loperamide until the subject is free from diarrhea for 12 hours.

For Grade 3 or 4 diarrhea or Grade 1 or 2 with complicating features (severe cramping, severe nausea/vomiting, decreased performance status, fever, sepsis, Grade 3 or 4 neutropenia, frank bleeding, dehydration) use intravenous fluids as appropriate, consider hospital administration. Use prophylactic antibiotics as needed (example fluoroquinolones) especially if diarrhea is persistent beyond 24 hours or there is a fever or Grade 3-4 neutropenia, hold both cytotoxic chemotherapy and lapatinib and discuss with medical monitor.

Treatment of gastrointestinal adverse events

Diarrhea can be debilitating, and on rare occasions, it is potentially life-threatening. Based on experience with lapatinib alone or in combination with taxanes and/or trastuzumab, diarrhea should be managed proactively to avoid complications or worsening of the patient's condition. Guidelines developed by an American Society of Clinical Oncology (ASCO) panel for treating chemotherapy-induced diarrhea are abstracted below.
Pharmacological approaches include the following:
Loperamide, administered as an initial 4-mg dose, followed by 2-mg doses every

4 hours. This dose and regimen are moderately effective.
Clonidine, non-steroidal anti-inflammatory drugs, and the serotonin antagonist cyproheptadine have been shown to be effective in controlling diarrhea associated with inflammation of the bowel.
The synthetic octapeptide, octreotide, has been shown to be effective in the control of diarrhea induced by fluoropyrimidine-based chemotherapy regimens when administered as an escalating dose by continuous infusion or subcutaneous injection. Octreotide can be administered at doses ranging from 100 µg twice daily to 500 µg 3 times daily, with a maximum-tolerated dose of 2000 µg 3 times daily in a 5-day regimen.

APPENDIX L: CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration

lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.