Title: Targeted therapy with lapatinib in patients with recurrent pituitary tumors resistant to standard therapy

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Section 1.0 General Information

Treatment of recurrent pituitary tumors with lapatinib
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Section 2.0 Background information

B1. erbB receptor tyrosine kinases.

The erbB receptor tyrosine kinase family consists of EGFR, erbB2, erbB3, and erbB4. They comprise an extracellular ligand-binding domain, a single membrane-spanning region and a cytoplasmic tyrosine-kinase-containing domain, and are expressed in epithelial, mesenchymal, and neuronal tissues. There are three groups of ligands for the erbB family. EGF, TGFα, amphiregulin, and others bind EGFR. The second group of ligands includes betacellulin, heparin-binding EGF, and epiregulin which bind both EGFR and erbB4. The third group comprises the neuregulins which bind erbB3 and erbB4 (Hynes et al. 2005). Ligand binding induces formation of receptor homo- and heterodimers and activation of the intrinsic kinase domain, leading to phosphorylation on specific tyrosine residues in the cytoplasmic tail. Phosphorylated residues serve as docking sites for proteins which lead to activation of intracellular signaling pathways (Hynes & Lane 2005).

erbB2 lacks a known direct ligand. erbB2 acts as a co-receptor or heterodimerization partner for other erbB receptors that possess stimulatory ligands. erbB2/erbB3 heterodimers are the most prevalent and mitogenically potent of the erbB receptor/ligand complexes (Roskoski 2004). Unlike other erbB receptors, erbB2 overexpression can cause malignant transformation without expression of a growth factor. erbB2 has constitutive (ligand-independent) activity, and erbB2 expression above a specific threshold can drive tumor growth (Yarden et al. 2001). erbB2 plays an important role in development in the nervous system, bone, muscle, skin, heart, and lungs, (Yarden & Sliwkowski 2001).

erbB3 possesses a tyrosine kinase domain homologous to the other members. However, kinase activity of erbB3 is impaired. erbB3 forms heterodimers with the other three members whereby *trans* phosphorylation is required for cell signaling (Roskoski 2004). erbB3 homodimers are mitogenically inactive (Pinkas-Kramarski et al. 1996).

erbB signaling is thus an interactive system in which the ligand can lead to formation of different homodimers or heterodimers that interact to stimulate a variety of signaling pathways. The network consist of an input layer (ligands or growth factors), cellular information processing layer (receptors, SH2-proteins, transcription factors), and an output layer (cell growth, differentiation, or migration). Receptor dimerization allows the signaling network to transmit biological messages with erbB2 as the coordinator of this intricate network (Yarden 2001).

B2. erbB receptor tyrosine kinase role in malignant transformation.

EGFR regulates cell proliferation and survival. An imbalance in this system can lead to increased EGFR signaling which can result in neoplastic transformation.

The role of EGFR overexpression in tumorigenesis includes amplification of the EGFR gene which then increases EGFR levels (Zandi et al. 2007). EGFR can also be

overexpressed in the absence of gene amplification from increased activity of the EGFR promoter or dysregulation at translational and post-translational levels. For instance, a specific region in the EGFR gene has an enhancer ability in some breast cancer lines overexpressing EGFR (McInerney et al. 2001). One mechanism involved in neoplastic transformation is aberrant EGFR signaling due to defective receptor downregulation. EGFR signaling is attenuated through internalization and subsequent degradation of the activated receptor. A mutant EGFR lacking part of the intracellular domain can transform mouse fibroblasts in a ligand dependent manner (Wells et al. 1990). EGFR downregulation can be affected by overexpression of erbB2. erbB2 is overexpressed in different cancers and can shift the formation of EGFR homodimers toward the formation of EGFR/erbB2 heterodimers. Activated erbB2 is not downregulated and when expressed together with EGFR at high levels, erbB2 can inhibit downregulation of EGFR (Qian et al. 1994). erbB2 overexpression leads to constitutive EGFR activation independent of ligand, with defective downregulation of erbB2-EGFR heterodimers leading to more potent signaling as compared with EGFR homodimers(Worthylake et al. 1999).

Dimerization of erbB receptors represents the fundamental mechanism that drives transformation. erbB2 forms heteromeric associations with EGFR and even without ligand, slight overexpression of erbB2 and EGFR led to heterodimers with activated kinases (Wada et al. 1990). erbB receptor dimerization triggers MAPK and PI3K-AKT pathways which then promote cell proliferation and survival (Zhang et al. 2007).

Over expression of erbB2 in tumors is a predominant transformation-activating mechanism. Over expression of erbB2 has been observed in 25-30% of breast and ovarian cancers, and is associated with poor prognosis (Slamon et al. 1987). This may be a consequence of gene amplification (Pauletti et al. 1996). erbB2 overexpression triggers ligand-independent activation of the kinase domain both from spontaneous dimer formation and heterodimerization. erbB2 oncogenic action occurs through many pathways and includes a dominant role of lipid kinase Phosphatidylinositol (PI)-3K pathway (Nelson et al. 2001). The ability of erbB2 to potentiate autocrine EGFR signaling may lead to activation of intracellular pathways, leading to increased proliferation and tumor development. Loss of erbB2 function inhibits proliferation of tumor cells displaying EGFR autocrine activation (reviewed in (Olayioye et al. 2000).

Transformation by either erbB2 or EGFR alone requires high receptor expression though expression of both receptors at moderate levels sufficient to cause transformation (Kokai et al. 1989) and depends on continuous cell surface expression of both receptors (Wada et al. 1990). Studies with the T757 mutant and N691stop mutant which has an intracellular truncation of erbB2 demonstrate that EGFR may be responsible for erbB2 phosphorylation and that erbB2 is involved in reciprocal activation of EGFR. Also, the mutations abolished transformation and tumorigenicity seen with formation of the wild type heterodimer (Qian et al. 1994). Overexpression leads to spontaneous receptor oligomerization, as with the EGFR receptor.

Expression of erbB3 is seen in a number of tumors which over express erbB2, including breast, bladder, and melanomas. Many erbB2 overexpressing breast tumors have elevated levels of phosphotyrosine on erbB3 likely as a result of spontaneous dimerization with erbB2. erbB2 and erbB3 function together to stimulate mitogenic signaling networks (reviewed in (Olayioye et al. 2000).

Nuclear EGFR localization has been observed in proliferating hepatocytes, pregnant uterus, and thyroid. Nuclear EGFR signaling network transmits growth factor signals directly from the cytoplasmic membrane to transcriptional targets in the nucleus, bypassing traditional protein phosphorylation cascades. The juxtamembrane region of EGFR harbors a putative nuclear localization sequence that mediates nuclear localization

of EGFR (Hsu et al. 2007). Recently, nuclear EGFR protein in oropharyngeal cancer tissue was shown to be associated with nuclear PCNA (Psyrri et al. 2008).

B3: EGFR targeted therapy in cancer

Knowledge of the role of the EGFR members in malignant transformation has led to development of a new class of therapeutics designed to interfere with this crucial mechanism. Two main types of therapies are monoclonal antibodies (mAb) targeting the extracellular domains and tyrosine kinase inhibitors (TKIs). mAb against EGFR inhibited EGF and nducted activation of EGFR tyrosine kinase activity and cell proliferation (Sato et al. 1983). Cetuximab (Erbitux) is a chimeric mouse anti-EGFR mAb approved for treatment of refractory colorectal cancer and in advanced head and neck cancer.

mAbs targeting erbB2 induce downmodulation of cell surface expression of the erbB2 receptor leading to reversal of the transformed phenotype of tumor cells (Drebin et al. 1985) as well as inhibiting growth of tumors overexpressing erbB2 implanted in athymic mice (Drebin et al. 1986). Trastuzumab (Herceptin) is approved for use in combination with first line chemotherapy for metastatic breast cancer expressing high levels of erbB2 (Zhang et al. 2007).

A key feature of erbB receptors is the inducible kinase activity that trans-phosphorylates tyrosine residues in the C terminal domain and leads to activation of downstream signaling pathways. Reversible inhibitors of this tyrosine kinase activity compete with the ATP binding to the kinase. TKIs have broad activity against multiple receptors in the erbB family (Zhang et al. 2007). Reversible TKIs include gefitinib (Iressa; AstraZeneca) approved for chemotherapy-refractory nonsmall cell lung cancer. It targets the EGFR tyrosine kinase specifically. Lapatinib (Tykerb, Glaxo) targets both EGFR and erbB2, leading to apoptosis of head, neck, and breast cancer cells (Xia et al. 2002). It is approved for use in combination with capecitabine for advanced erbB2-positive breast cancer (Cameron et al. 2008).

B4. Natural history of pituitary tumors.

Pituitary adenomas comprise ~15 % of intracranial neoplasms with overall incidence at 15 to 20 per million per year. The prevalence of clinically significant adenomas is between 2 to 2.5 per 10,000 though this may be up to 9 per 10,000 in other series (Levy 2008). Occult adenomas are discovered in as many as 25 % of unselected autopsies (Melmed 2003). Microadenomas (less than 1 cm diameter) comprise 50-60 % of these and tend to show no further growth. Macroadenomas slowly expand over years. Pituitary carcinomas comprise less than 0.2 % of pituitary adenomas (Levy 2008). Pituitary tumors are generally benign but can lead to morbidity from their location near critical structures, increasing size, and pituitary hormone over- or under-expression. Patients may experience headaches, visual disorders, and cranial nerve dysfunction from compressive effects while changes in hormone expression may either be due to pituitary stalk disruption or pituitary failure from compression of normal pituitary tissue. Pituitary adenomas produce clinical features based on their specific cell type. Prolactinomas comprise about 30 % of pituitary adenomas with a prevalence of 60-100 per million and present with amenorrhea, infertility, and galactorrhea in females and impotence or infertility in males. Somatotroph adenomas comprise 10 % of pituitary adenomas with prevalence of 40-60 per million and overexpress growth hormone leading to acromegaly in adults as manifested by soft tissue and bony changes and increased risk of hypertension, cardiac disease, and diabetes. Another 10 % of adenomas are corticotroph adenomas which lead to ACTH hypersecretion and features of hypercortisolism. Thyrotropinomas comprise are rare (1 % of adenomas). Approximately 30-35 % of tumors are nonfunctioning and present generally with compressive symptoms and

hypopituitarism. The prevalence is 70-90 per million and include the subsets of gonadotroph adenomas, oncocytic and non-oncocytic adenomas (Melmed 2003).

Though pituitary adenomas are usually benign, some exhibit aggressive growth patterns and may invade parasellar structures, recur and progressively grow despite antitumor therapy, as well as even metastasize to intracranial and other distant sites (Blevins et al. 1998). There is no accepted definition of aggressive adenomas but are generally observed to involve massive invasion of surrounding brain and parasellar anatomy, rapid growth, and giant size. They tend to recur quickly after initial treatments, are unresponsive to therapy, difficult to cure, and some have a fatal outcome. Invasion into the cavernous sinus, skull base bone, and sphenoid sinus mucosa prevents curative surgery in these tumors (Buchfelder 2008).

Radiologic signs of invasion include: a tumor that completely encircles or extends beyond the lateral limit of the cavernous carotid artery; complete erosion of the clivus; and the presence of tumor within the sphenoid sinuses. Dural microinvasion occurs in 69 % of microadenomas and 94 % of macroadenomas(Selman et al. 1986).

The pathogenesis of pituitary carcinomas is unclear. They may arise from preexisting adenomas as shown by the fact that many carcinomas initially present as macroadenomas, demonstration of a monoclonal origin, and confirmation of the progressive accumulation of genetic alterations in the transformation from a pituitary adenoma to a carcinoma. Invasive adenomas may represent a biologically intermediate stage in tumor progression from benign to carcinoma (Amar et al. 1999). According to the 2004 WHO classification, tumors with invasive growth, elevated mitotic index, Ki-67 labeling index > 3 %, and extensive nuclear reactivity for p53 are considered “atypical” adenomas warranting closer monitoring for development of early regrowth (Al-Shraim et al. 2006).

The standard therapy for nonfunctioning adenomas is surgical resection as there are no effective medical therapies to date. Surgical success is dependent on the skill and experience of the neurosurgeon and on degree of invasion and size. Tumors larger than 2 cm can be completely removed in 20 % of cases while invasion into the cavernous sinus makes it difficult to resect (Chandler et al. 2008). Completeness of resection is assessed on the 3 month postoperative MRI scan and annual serial MRIs. Preoperative hypopituitarism may resolve in 15-50 % of patients and hyperprolactinemia may improve in up to two-thirds of patients. However, surgery can also lead to development of new onset hypopituitarism in 2-15 % of patients. Transient diabetes insipidus (DI) may occur in 33 % of patients with permanent DI in 0.5-5 %. Other complications of surgery include cerebrospinal fluid leak, meningitis, rebleeding, cranial nerve injury, visual field compromise. Mortality is 0.3-0.5 % with higher mortality and morbidity in large tumors (> 4 cm diameter) (Molitch 2008). Reoperation for recurrences is often necessary in nonfunctioning adenomas when there is risk to the optic apparatus. Each additional surgery increases the risks of bleeding and further damage to the normal pituitary and surrounding structures.

In contrast to nonfunctioning adenomas, prolactin secreting adenomas are treated with primary medical therapy through use of dopamine agonists. Bromocriptine normalizes prolactin levels in 80-90 % of microprolactinomas and in 70 % of macroprolactinomas. In 20 % of cases, patients will be resistant to medications and fail to normalize prolactin levels and shrinkage of tumor mass on MRI. In those cases, surgery is indicated with 75 % cure rate in microprolactinomas and 34 % cure rate in macroprolactinomas(Mancini et al. 2008).

B5. Pituitary tumorigenesis.

Pituitary adenomas arise from clonal expansion of mutated somatic cells. Loss of heterozygosity of the chromosomal locus, 11q13, has been observed in 30 % of sporadic pituitary tumor, especially the invasive types (Melmed 2003). p27 protein is absent in most pituitary tumors and undetectable in pituitary carcinomas (Lidhar et al. 1999). Disruption of p18, a cyclin inhibitor gene, and p27 lead to development of pituitary tumors with accelerated tumorigenesis(Franklin et al. 1998). p16 is undetectable in pituitary tumors and may be inactivated in adenomas (Melmed 2003). Approximately half of pituitary adenomas are aneuploid(Levy et al. 2003) but the gain or loss of chromosomes has been inconsistent (Hui et al. 1999). Cytologicatypia does not correlate with rapid tumor growth though the presence and number of mitoses may be a possible indicator (Kontogeorgos 2006). p53 immunoreactivity is absent in noninvasive adenomas while present in 15 % of invasive adenomas and in all pituitary carcinomas (Kontogeorgos 2006). Apoptotic reactivity has been observed in aggressive, atypical, and drug-resistant pituitary adenomas (Kontogeorgos et al. 2006).

PTTG is over expressed in pituitary tumors and correlates with invasiveness, behaving as a transactivator and potent transforming gene (Pei 2000). PTTG disruption in mouse models is protective for pituitary tumor development (Chesnokova et al. 2005) and associated with pituitary cell senescence thereby restraining development of pituitary tumors (Chesnokova et al. 2007). Apoptosis and senescence control aberrant tissue growth and contribute to tumor suppressor activity. Cellular senescence is characterized by up-regulation of cell cycle progression inhibitors, p19, p21, and p16. PTTG may behave as a protooncogene depending on pituitary p21 status. High levels of PTTG lead to chromosomal instability and defective metaphase-anaphase progression and promote pituitary tumor formation. Activation of pituitary DNA damage pathways triggers p21, a barrier of tumor growth and marker of senescence, which may then restrain further growth and malignant transformation of pituitary tumors. When the senescence pathway is bypassed, malignant transformation of pituitary adenomas may then occur. This has been supported by presence of p21 expression in > 70 % of GH secreting adenomas and absence of p21 in GH carcinomas (Chesnokova et al. 2008).

B6. erbB receptor tyrosine kinases in pituitary tumors.

Crosstalk between PRL and EGF was observed in T47D cancer cells, PRL activated JAK2, STAT5, and ERKs and caused phosphorylation of EGFR and erbB2. PRL synergized with EGF in activating ERK (Frank 2008).

erbB receptors are over expressed in pituitary adenomas. In 20 nonfunctioning adenomas, 80 % were immunopositive for EGFR and all were erbB2 positive while EGFR was undetectable and erbB2 weaker in functioning tumors (Chaidarun et al. 1994). Another study found 50 % of functioning adenomas expressed EGFR while most nonfunctioning adenomas and normal pituitary expressed EGFR. EGFR mRNA expression was highest in somatotroph adenomas and in patients with aggressive silent subtype 3 adenomas and with recurrent acromegaly (LeRiche et al. 1996). In contrast, others found that nonfunctioning adenomas were uniformly negative for EGFR while 50 % of functioning tumors were positive (Kontogeorgos et al. 1996). More recently, EGFR expression was shown in normal pituitaries and nonfunctioning adenomas with lowest expression in corticotroph adenomas. *In situ* hybridization for EGFR was positive in 77 % of null cell and 68 % of gonadotroph adenomas with variable positivity in functioning adenomas. Five pituitary carcinomas though were strongly positive with one tumor negative. Western blot analysis confirmed expression in nonfunctioning adenomas and carcinomas (Onguru et al. 2004). Furthermore, EGF binding was higher in invasive

adenomas than in non-invasive adenomas, especially in those invading the sphenoidal sinus (Jaffrain-Rea et al. 1998). However, only 3 of 40 nonfunctioning adenomas were immunopositive for EGFR in contrast to 33 % of prolactinomas and 25 % of somatotroph adenomas though 80 % of corticotroph adenomas were positive (Theodoropoulou et al. 2004).

erbB2 is expressed in 67 % prolactinomas and in 53 % of somatotropinomas(Botelho et al. 2006). erbB2 staining was observed in a pituitary corticotroph carcinoma but not in corticotroph adenomas (Nose-Alberti et al. 1998). In a gonadotroph carcinoma, erbB2 immunoreactivity and gene amplification were manifest in the recurrent tumor (Roncaroli et al. 2003). Another study did demonstrate no erbB2 immunostaining or gene amplification of erbB2 in pituitary adenomas .

C. Preliminary Data:

C1. EGFR-mediated pituitary signaling *in vitro* and *in vivo*

EGFR and erbB2 are expressed in GH3 rat lacto-somatotroph cells but not in AtT20 mouse corticotroph cells. Our laboratory showed that EGF treatment enhanced baseline and serum-induced prolactin mRNA and gene expression in GH3 cells but did not change AtT20 POMC gene expression (figure 1). Treating GH3 cells with increasing doses of gefitinib attenuated serum induced S phase entry and suppressed serum induced Pttg1 mRNA expression. Gefitinib, an EGFR inhibitor, inhibited prolactin mRNA expression as well. Doses as low as 0.1µmol/L led to a 15 % attenuation of GH3 cell number with a dose dependent decrease of 72 % at 10 µmol/L. When GH3 cells were pretreated with gefitinib prior to induction with EGF, prolactin gene expression was suppressed (Vlotides et al. 2008).

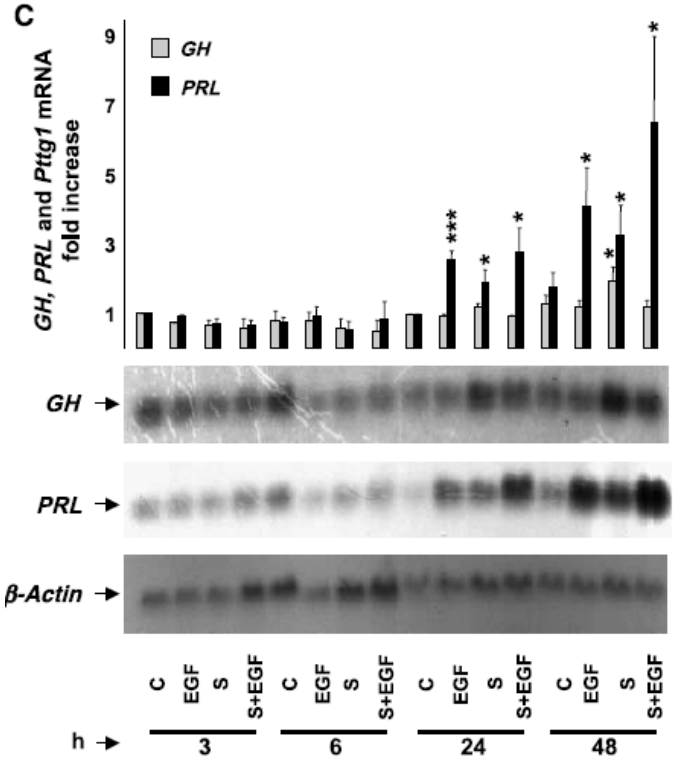


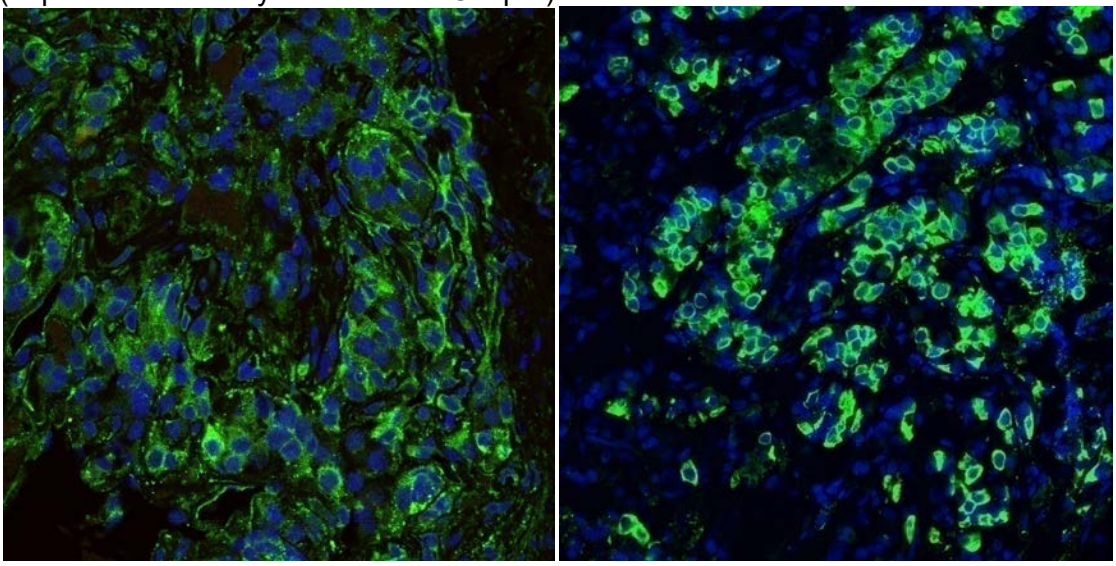
Figure 1: EGF selectively induces PRL secretion and mRNA levels. PRL and GH mRNA levels on Northern blot at different time points. C: control. EGF: GH3 cells treated with 5 nmol/L. S: serum. *p < 0.05, *** p < 0.001

(Vlotides et al. 2008)

Induction of GH3 cells with EGF or serum induced tyrosine phosphorylation of erbB receptor members. This was confirmed by immunoprecipitation with receptor-specific antibodies. EGF induced tyrosine phosphorylation of EGFR which was blocked by gefitinib pretreatment. GH3 cell *neu* tyrosine phosphorylation was seen in response to ligand activation. Tyrosine phosphorylation of erbB2 was observed in response to EGF and heregulin, confirming that erbB2 is the preferred erbB family heterodimerization partner in GH3 cells. Gefitinib treatment abrogated EGF induced erbB2 activation. erbB3 tyrosine phosphorylation was seen in response to heregulin but in low levels in response to EGF (Vlotides et al. 2008).

GH3 cells were implanted into athymic mice. In gefitinib-treated animals, tumor volume decreased by 50 % compared to controls. Prolactin and IGF-1 levels as well decreased significantly. Immunohistochemical analysis confirmed EGFR expression in tumor tissue (Vlotides et al. 2008).

We show immunofluorescence staining for erbB2 and erbB3 in 7 prolactinomas (figure 2). mRNA expression for erbB2 and erbB3 by quantitative PCR analysis of two tumor specimens was elevated. In a patient with a prolactinoma resistant to treatment who had multiple surgeries, higher erbB2 and erbB3 levels were seen in the recurrent tumor. In rat lacto-somatotroph GH4C1 cells, immunoprecipitation and immunoblotting showed expression of EGFR, erbB2, and erbB3. EGF induced EGFR and erbB2 phosphorylation but not of erbB3. Increasing heregulin concentrations induced PRL mRNA expression. Gefitinib treatment suppressed receptor activation and signaling of erbB receptors and prevented heregulin-induced erbB2/erbB3 heterodimerization (unpublished data by Vlotides and Cooper).



A. **B.**

Figure 2: Immunohistochemistry of human prolactinoma A. erbB2 expression (SC-284) B: erbB3 (SC-284) expression

C2. Lacto-somatotroph stable transfectants.

A GH4 cell line was engineered in our laboratory to constitutively overexpress erbB2. This cell line increased prolactin expression 1.5 fold compared to controls. In contrast, GH4 cells with kinase negative erbB2 expression demonstrated lower PRL gene expression (figure 3-4) while GH expression was unchanged. These stable transfectants will be used for *in vivo*tumorigenesis experiments (see below).

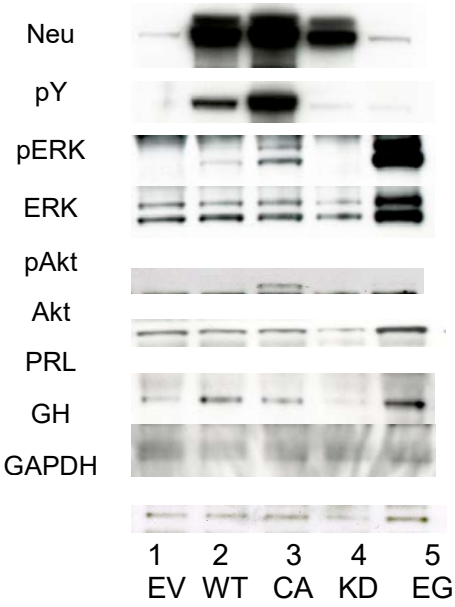


Figure 3: erbB2 induced PRL expression in GH4 cells. Cells were stably transfected with empty vector, wild type erbB2, constitutively active erbB2 or kinase deficient erbB2. Lane1: empty vector. Lane 2: erbB2 wild type in GH4 cells. Lane 3: constitutively active erbB2 in GH4 cells. Lane 4: kinase deficient erbB2 in GH4 cells. Lane 5: EGF treated cells.

C3. Expression of erbB receptors in invasive versus non-invasive pituitary adenomas.

We evaluated 23 large NFAs and 13 small NFAs in a cohort analysis using immunohistochemistry and immunoblotting to test for EGFR, erbB2, and erbB3. Fisher's exact test was used for categorical variables, and the t-test and Mann-Whitney test used to compare means of continuous variables.81 % of large NFAs were males while in the small NFAs 20 % were males (p=0.0013). Preoperative presentation in large NFAs differed in visual field loss (p = 0.0136) and secondary hypogonadism (p=0.023). Median tumor MRI diameter in large NFAs was 40 ± 17 mm and 16 ± 6 mm in small NFAs (p=0.0002). 65 % of large NFAs had cavernous sinus invasion (p=0.0005), 96 % had optic chiasm compression (p=0.04), 40 % had sphenoid sinus invasion (p =0.0116), 81 % (p=0.03) had suprasellar tumor, 35 % had sphenoid invasion (p=0.03), and 53 % had extralobar spread (p=0.03). Small NFAs underwent 1 surgery while 56 % of large NFAs had more than one surgery (p=0.0451). 76 % of large NFAs had residual tumor (p=0.007) and 56 % had recurrences (p=0.0451) compared to none in small NFAs (unpublished data, Cooper O).

Positive staining for EGFR in large NFAs was 93 ± 4 % (mean) of tumor cells vs 68 ± 11% in small NFAs (p<0.0001), and positive staining for erbB2 in large NFAs was 93 ± 4 % (mean) of tumor cells for erbB2 versus 81 ± 15 % in small NFAs (p=0.0004). Both large and small adenomas had nuclear localization of EGFR as opposed to membranous staining of erbB2. Membranous erbB3 staining was present in 42 % of large NFAs compared to 33 % of small NFAs (figure 5). Western blot confirmed expression of EGFR, erbB2, and erbB3 in 3 large adenomas and none in a small adenoma (figure 6). EGFR staining correlated with increasing tumor diameter (p=0.0004), suprasellar spread (p=0.017), cavernous sinus invasion (p=0.056), extralobar spread (p=0.011), residual tumor after surgery (p=0.0077), and recurrences (p=0.05). erbB2 staining correlated with suprasellar invasion (p=0.045). Large NFAs had 37 % higher percentage of cells

expressing EGFR and 15 % higher erbB2 expression than small NFAs (unpublished data, Cooper O).

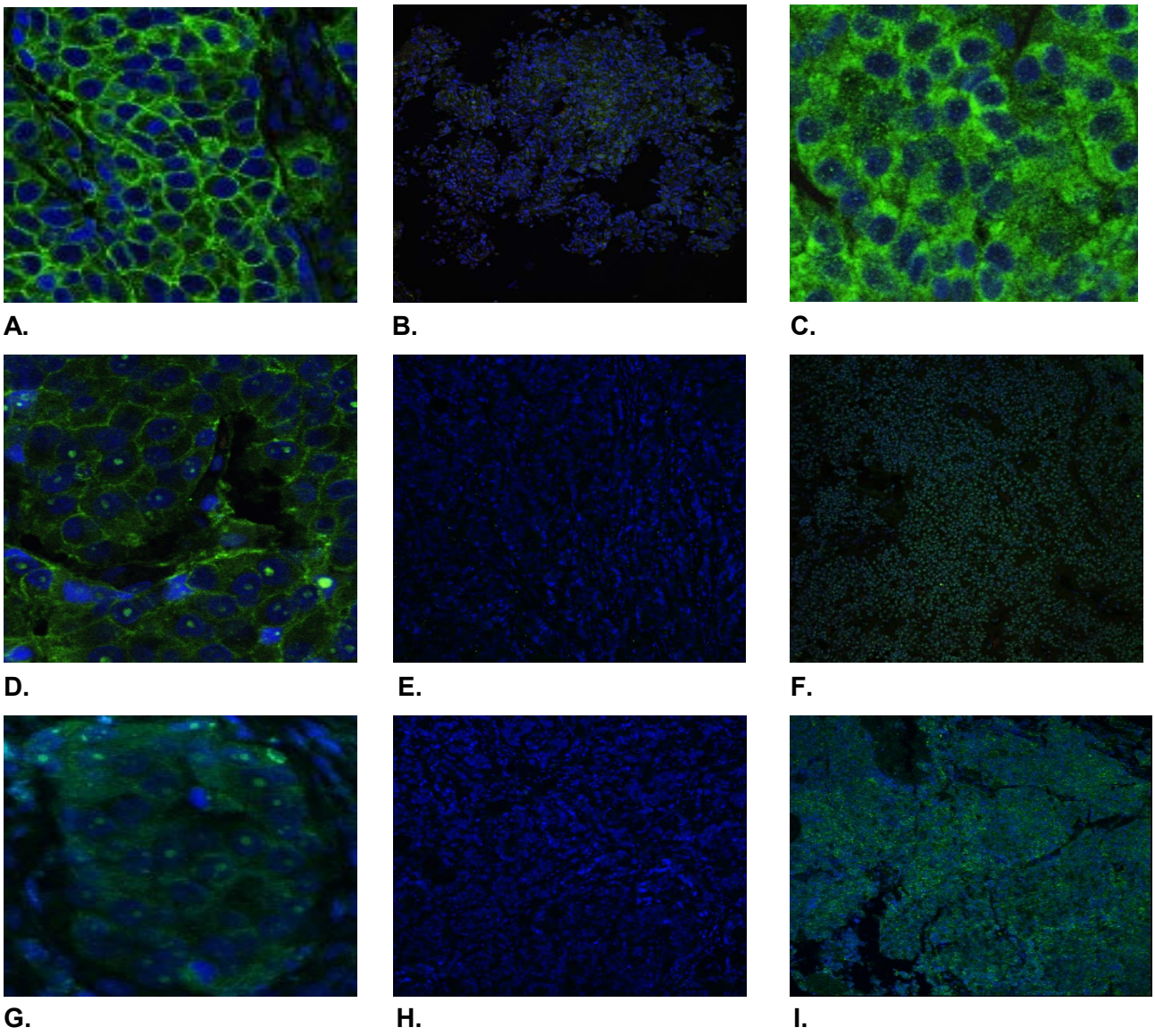


Figure 5: Immunofluorescent staining of giant nonfunctioning human pituitary adenomas (NFAs). A: erbB2 in breast cancer (positive control). B: Negative control for erbB2 (corticotroph tumor) C. erbB2 in giant NFA. D: EGFR in breast cancer. E. EGFR blocking peptide of breast tissue. F. EGFR in giant NFA. G: erbB3 in breast cancer. H. erbB3 blocking peptide of breast tissue.I. erbB3 in giant NFA.

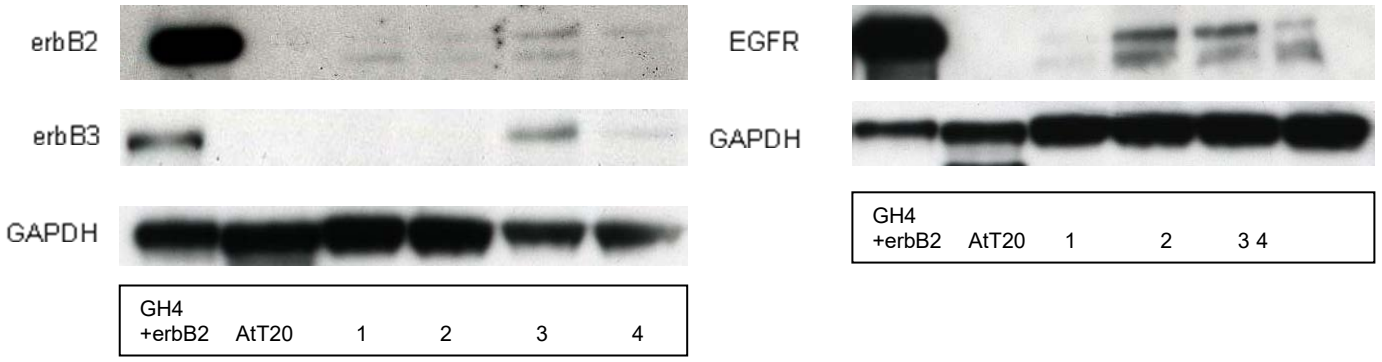


Figure 6: erbB is overexpressed in human NFAs as assessed by Western blot. Positive controls for erbB2, erbB3, and EGFR: GH4 erbB2 overexpressing cells; negative controls: AtT20 cells; Tumors 1 and 2: small NFAs, Tumors 3 and 4: large NFAs

Summary:
We have demonstrated expression of EGFR and erbB2 in rat lacto-somatotroph cells, and EGF stimulation leads to increased prolactin mRNA expression. In GH4 cells which overexpress constitutively active erbB2, prolactin expression increased. Gefitinib treatment inhibits EGF induced erbB2 and EGFR in GH3 cells, and in mice implanted with GH3 cells, lead to decreased tumor volume and prolactin levels. Expression of the erbB receptors has been shown in human prolactinomas resistant to medical therapy as well as in large, invasive nonfunctioning adenomas.

Section 3.0 Trial objectives and purpose

Test the clinical effects of lapatinib for treatment of aggressive and recurrent pituitary tumors.

Hypothesis: We hypothesize that lapatinib will inhibit tumor growth and hormonal secretion in patients with pituitary tumors. Gefitinib has been approved for use in non-small-cell lung cancer but has not been proven to elicit significant tumor size reduction. Lapatinib, which inhibits both EGFR and erbB2 tyrosine kinases, has been proven to be effective in slowing disease progression in breast cancer both as monotherapy and when given in combination with capecitabine(Geyer et al. 2006). In a proof of concept clinical trial, we plan to treat patients with aggressive pituitary tumors resistant to standard therapy with lapatinib for 6 months to assess for stabilization of tumor size and pituitary tumor secretory profiles. In addition, we will treat subjects with malignant pituitary tumors which have failed to respond to other therapeutic modalities. These subjects will be in a separate arm of the study for patients with malignant pituitary tumors.

Section 4.0 Trial Design

Conduct a proof of concept single cohort open label study of lapatinib in patients with pituitary tumors.

Trial design: Open label, single cohort proof of concept trial.
An additional arm of the study will be for subjects treated with the study drug in malignant pituitary tumors.
Three clinical trial sites, one at CSMC and the others at Johns Hopkins Hospital and Massachusetts General Hospital.

Study population: Patients will be recruited from the Pituitary Center at Cedars-Sinai Medical Center and the other two sites.
Intervention: lapatinib 1250 mg daily for 6 months. Patients will be treated with the standardized dose used in breast cancer therapy for six months (Cameron & Stein 2008; Geyer et al. 2006). The drug will be supplied by Novartis Oncology.

a. Primary outcome variable.

Primary outcome in nonfunctioning pituitary adenomas: 40% reduction in tumor size in any dimension. .Tumor size and volume will be calculated using Open Source OsiriX® Imaging Software for MacOS. MRIs will be performed at baseline and at 3 and 6 months All MRIs will be centrally read by the dedicated pituitary radiologist at CSMC.

Primary outcome in prolactinomas: 40% reduction in tumor size in any dimension.
Primary outcome in malignant pituitary tumors: Progression free survival

b. Secondary outcome variables.

Secondary outcomes in prolactinomas:
(a) achievement of 50% reduction of PRL levels (b) PRL normalization (c) percent reduction in PRL levels (d) percent reduction in tumor volume. Tumor volume will be calculated using Open Source OsiriX® Imaging Software for MacOS. (e) correlation of tumor size and PRL reduction (f) clinical predictors of TKI response, including age, gender, duration of disease, dose and duration of dopamine agonist therapy, baseline PRL, and baseline tumor size.

Secondary outcomes in malignant pituitary tumors: partial response, complete response, mortality, 24 hour urine free cortisol levels for Cushing carcinomas, IGF-1 levels for acromegaly carcinomas.
We will correlate ErbB receptor expression with the subjects' clinical response to the study drug. This will be performed through immunohistochemistry staining of pituitary tumor slides from surgery.

Section 5.0. Selection and Withdrawal of Subjects

Inclusion criteria: Subjects with non-functioning pituitary adenomas, who have undergone at least one prior surgical resection and have demonstrated recurrence on MRI.
Subjects with prolactinomas who are resistant to dopamine agonist therapy who manifest continued tumor size growth while on a dopamine agonist, unless intolerant of adequate doses of a dopamine agonist.
Subjects with malignant pituitary tumors will be eligible for the malignant pituitary tumor arm of the study regardless of existing visual deficits.
Exclusion criteria: Children will be excluded. If subjects have preexisting visual field deficits, they will be monitored on more frequent basis. Patients will also be excluded if they have left ventricular ejection fraction less than 50 %, moderate to severe hepatic impairment (see below for specifics), active hepatitis, HIV positivity, concurrent cancers, life expectancy less than one year, and pregnancy. Pregnant patients will be excluded. Childbearing age women will be require to use

contraception. Patients who become pregnant during the study should discontinue the study immediately.

Withdrawal of Subjects:
Monitoring for adverse effects: Prior to initiating therapy, patients will undergo an echocardiogram and ECG to evaluate the ejection fraction, and the echocardiogram will be repeated every 8 weeks (Visit 3, 5, and 7) and on visit 8. Patients will also undergo ophthalmic testing at visits 1, 4, and 7.Patients with pre-existing stable visual field compromise prior to enrollment will undergo visual field testing at visits 1, 2, and 3,and if stable, then they will proceed with visual field testing every three months for the duration of the study. Patients will be evaluated for adverse effects monthly with physical examinations, chemistry and hematology panels, and ECGs. Adverse events will be graded by the NCI-CTC and include: anorexia, decreased ejection fraction, interstitial lung disease/pnemonitis, diarrhea, nausea, vomiting, hepatotoxicity, rash, and fatigue. Lapatinib will be discontinued in those who developed decreased ejection fraction, who develop pulmonary symptoms, retinal toxicity, worsening of visual fields, and those with toxicity greater than or equal to Grade 2 on the NCI-CTC. Dr. Jeremy Rudnick, from Oncology department at Cedars-Sinai Medical Center, will assist us in monitoring patients for adverse events.
If at any time a patient demonstrates compromise of his visual fields, dilated retinal exam or tumor growth on MRI, he will be withdrawn from the study and referred for surgery.

Section 6.0 Treatment of Subjects

Patients with recurrent nonfunctioning pituitary tumors or prolactinomas resistant to medical therapy typically undergo surgical resection of the tumor once the tumor demonstrates radiological progression associated with clinical signs and symptoms and/or optic chiasm compression. Otherwise, we observe tumor growth with serial MRIs as long as the chiasm is not compressed until surgery is deemed necessary for decompression of the optic chiasm. In our study, we are excluding any subjects who have compression of the optic chiasm and only will recruit those whom we would otherwise observe with serial MRIs to monitor the tumor growth. After completion of their participation in the study, patients will then resume the standard monitoring of tumor growth. However, if a given patient responds to the study drug and chooses to stay on the drug after cessation of the trial, clinical data will continue to be collected on the patient for further analysis of response to the drug. This will continue until they discontinue to the drug to monitor for safety.

Patients with prolactinomas who are currently being treated by dopamine agonist and patients with malignant tumors on dopamine agonists, somatostatin analogs, and growth hormone receptor antagonist medications prior to enrollment will remain on their drug therapy while on the study drug.

Patients will have a screening visit (visit 1) to determine eligibility for the trial and to assess for exclusion criteria. If the patient meets study criteria for enrollment, he will begin the study drug and be followed in subsequent visits. Study staff will call patients weekly for the first month on the study drug and then every 2 weeks for the second month on the study drug.
The following tests will be done as follows:

On baseline visit 1: time 0
History and physical exam

Draw screening labs: CBC with differential, BMP, LFTs (15 cc blood)
For those with acromegaly from malignant tumors, will GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Pituitary hormone profile: PRL, TSH, thyroid panel, free T4, IGF-1, LH, FSH, testosterone (total and free) for males, 8 am cortisol if indicated (total of 15 cc of blood)
Tubes of blood for lab to be frozen (to be used to measure EGFR and Her2 levels) (10 cc)
ECG
Echocardiogram
Check Visual Fields
Dilated retinal exam
Visual acuity check
Ocular pressure check
Color vision test
Urine Pregnancy Test
MRI—this will be waived if subjects had an MRI performed at their outside hospital within 3 months of the 1st study visit and if subjects provide a CD of the images for review by our neuroradiologist to determine if of sufficient quality for a baseline study.

While on the drug, visits will occur once a month to monitor for adverse effects. Patients' visits may not deviate more than 7 days from the 30-day mark between each visit.

Visit 2: time 1 month on drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with prolactinomas, will also check PRL.
For those with acromegaly from malignant tumors, will GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Do ECG
Urine Pregnancy Test
Those with pre-existing visual field compromise, visual field testing

Visit 3: time 2 months on drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with prolactinomas, will also check PRL.
For those with acromegaly from malignant tumors, will check GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Those with pre-existing visual field compromise, visual field testing
Do ECG, echocardiogram
Urine Pregnancy Test

Visit 4: time of 3 months on drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with prolactinomas, will also check PRL.
For those with acromegaly from malignant tumors, will check GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Pituitary hormones as well—TSH, thyroid panel, free T4, 8 am cortisol (if indicated), IGF-1, LH, FSH, testosterone (15 cc blood)
Tubes for lab for EGFR, her2 levels (10 cc blood)

Do ECG
Visual field test
Dilated retinal exam
Color Vision test
Do MRI of pituitary with and without IV gadolinium
Urine Pregnancy Test

Visit 5: time of 4 months on drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with prolactinomas, will also check PRL.
For those with acromegaly from malignant tumors, will check GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Do ECG, echocardiogram
Urine Pregnancy Test

Visit 6: time of 5 months on drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with prolactinomas, will also check PRL.
For those with acromegaly from malignant tumors, will check GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Do ECG
Urine Pregnancy Test
Those with pre-existing visual field compromise, visual field testing

Visit 7: time of 6 months on drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with acromegaly from malignant tumors, will check GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Pituitary hormones as well—PRL, TSH, thyroid panel, free T4, 8 am cortisol (if indicated), IGF-1, LH, FSH, total and free testosterone for males (15 cc blood)
Tubes for lab for EGFR, her2 levels (10 cc blood)
Do ECG, Echocardiogram
Check visual fields
Dilated retinal exam
Visual acuity check
Ocular pressure check
Color Vision test
Do MRI of pituitary with and without IV gadolinium
Urine Pregnancy Test

Visit 8: time of 1 mo off drug
Physical exam and history
Will draw CBC with differential, BMP, LFTs (15 cc blood)
For those with prolactinomas, will also check PRL.
For those with acromegaly from malignant tumors, will check GH levels on 2 hour OGTT
For those with ACTH secreting malignant tumors, will check 24 hour urinary free cortisol
Draw tubes for EGFR, Her2 levels (10 cc blood)
Do ECG, Echocardiogram

Urine Pregnancy Test

Note: Study visits may be conducted over 2 days to allow for sufficient time to complete the procedures, scheduling availability, and patient preference.
Study procedure flowsheet:
(See following page)

Procedure	**Baseline Visit**	**Visit #2 (+30 days)**	**Visit #3 (+60 days)**	**Visit #4 (+90 days)**	**Visit #5 (+120 days)**	**Visit #6 (+150 days)**	**Visit #7 (+180 days)**	**Visit #8** (+210 days)**
Laboratory Blood Tests (may include levels of hormones, cholesterol, CBC, and metabolic panel)	X	X	X	X	X	X	X	X
MRI of the Pituitary	X^^			X			X	
Echocardiogram	X		X		X		X	X
Electrocardiogram (ECG)	X	X	X	X	X	X	X	X
Visual Field Test	X	X ^	X ^	X		X ^	X	
History and Physical	X	X	X	X	X	X	X	X
Informed Consent	X							
Color Vision Test	X			X			X	
Ocular Pressure Check	X						X	
Visual Acuity	X						X	
Dilated Retinal Exam	X			X			X	
Hormonal profile	X	X	X	X	X	X	X	X
Oral Glucose Tolerance Test (OGTT) (malignant arm)	X*	X*	X*	X*	X*	X*	X*	X*

24-hour Urinary Free Cortisol (malignant arm)	X+	X+	X+	X+	X+	X+	X+	X+
Pregnancy Test for pre-menopausal women	X	X	X	X	X	X	X	X

^ Only for subjects with visual field deficits
^^ will be waived if subjects had MRI at outside hospital within 3 months of baseline visit
* Only for subjects with acromegaly from malignant tumors
+Only for subjects with ACTH secreting adenomas from malignant tumors
**Visit 8 is only required if stopping Lapatinib at visit 7. Patients continuing on Lapatinib after visit 7 will be followed by their physician as clinically appropriate.

Section 7.0 Assessment of Efficacy

a. Primary outcome variable.

Primary outcome in nonfunctioning pituitary adenomas: 40% reduction in tumor size in any dimension. Tumor size and volume will be calculated using Open Source OsiriX® Imaging Software for MacOS. MRIs will be performed at baseline and at 3 and 6 months All MRIs will be centrally read by the dedicated pituitary radiologist at CSMC.
Primary outcome in prolactinomas: 40% reduction in tumor size in any dimension.
Primary outcome in malignant pituitary tumors: Progression free survival

b. Secondary outcome variables.

Secondary outcomes in prolactinomas:
(a) achievement of 50% reduction of PRL levels (b) PRL normalization (c) percent reduction in PRL levels (d) percent reduction in tumor volume. Tumor volume will be calculated using Open Source OsiriX® Imaging Software for MacOS. (e) correlation of tumor size and PRL reduction (f) clinical predictors of TKI response, including age, gender, duration of disease, dose and duration of dopamine agonist therapy, baseline PRL, and baseline tumor size.

Secondary outcomes in malignant pituitary tumors: partial response, complete response, mortality, 24 hour urine free cortisol levels for Cushing carcinomas, IGF-1 levels for acromegaly carcinomas
We will correlate ErbB receptor expression with the subjects' clinical response to the study drug. This will be performed through immunohistochemistry staining of pituitary tumor slides from surgery.

Section 8.0 Assessment of Safety

1. *Monitoring for adverse effects (further detailed below):* Data has been collected on over 12000 subjects on combination therapy of lapatinib and other chemotherapies, and over 4000 subjects have been treated with lapatinib monotherapy, with 2347 of those specifically in breast cancer. With monotherapy, majority of AEs were Grade 1 or 2 in severity. The incidence of Grade 3 and Grade 4 events was 21% in those

who received lapatinib and 7% in those on placebo. Risks of adverse events are not additive or cumulative. SAEs were reported in 6% of those on lapatinib compared to 5% on placebo. Cardiac events including left ventricular ejection fraction decreases were reported in approximately 1% of patients and most have partial or full recovery. Symptomatic decreases in ejection fraction were observed in approximately 0.3% of patients on lapatinib. Interstitial lung disease is observed in 0.2% of patients. Hepatotoxicity has been observed in <1% of patients. Grade 3 and 4 diarrhea occurs in <10% of patients. Nausea may be seen in 18% of patients and rash in 29% (Investigators' Brochure).

2. Prior to initiating therapy, patients will undergo an echocardiogram and ECG to evaluate the ejection fraction, and the echocardiogram will be repeated every 8 weeks and on visit. Patients will be evaluated for adverse effects monthly with physical examinations, chemistry and hematology panels, and ECGs. Adverse events will be graded by the NCI-CTC. Common adverse events include: hand-foot syndrome, rash, diarrhea, indigestion, nausea, vomiting, anorexia, anemia, low platelets, abnormal livers tests (ALT (SGPT) level raised, AST/SGOT level raised, hyperbilirubinemia, backache, pain in limb, insomnia, fatigue, and shortness of breath. Rare but serious events include: heart failure (depression of left ventricular systolic function), abnormal heart beat (prolonged QT interval), liver damage (hepatotoxicity), and lung problems (interstitial lung disease/pneumonitis). Lapatinib will be discontinued in those who developed decreased ejection fraction, who develop pulmonary symptoms. Those subjects with toxicity less than or equal to Grade 2 on the NCI-CTC will have their dose held and resumed when the toxicity has resolved. Those subjects with toxicity equal to Grade 3 on the NCI-CTC will have their dose held and resumed at a fifty percent dose reduction when the toxicity has resolved. Those subjects with toxicity equal to Grade 4 on the NCI-CTC will be discontinued on the study drug. Dr. Jeremy Rudnick, from Oncology department at Cedars-Sinai Medical Center, will assist us in monitoring patients for adverse events.
3. If at any time a patient demonstrates a worsening of their visual fields or tumor growth on MRI, they will be withdrawn from the study and referred for surgery.
4. For further safety monitoring, subjects may be asked to have additional laboratory testing as deemed necessary by the investigators. This would require subjects to have extra blood draws which can also be done at outside laboratories.
5. A DSMB will be set up to monitor safety of the study at all trial sites.

Cardiac Events
Addition to SAE definition:
Cardiovascular events have been seen in 1.0% of subjects on lapatinib with or following anthracyclines, with symptomatic events in 0.3%. As a precaution, the following will be reported as an SAE:

- 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 left ventricular cardiac ejection fraction (LVEF) relative to baseline which is below the institution's lower limit of normal.

Refer to NCI CTCAE grading of left ventricular cardiac function.

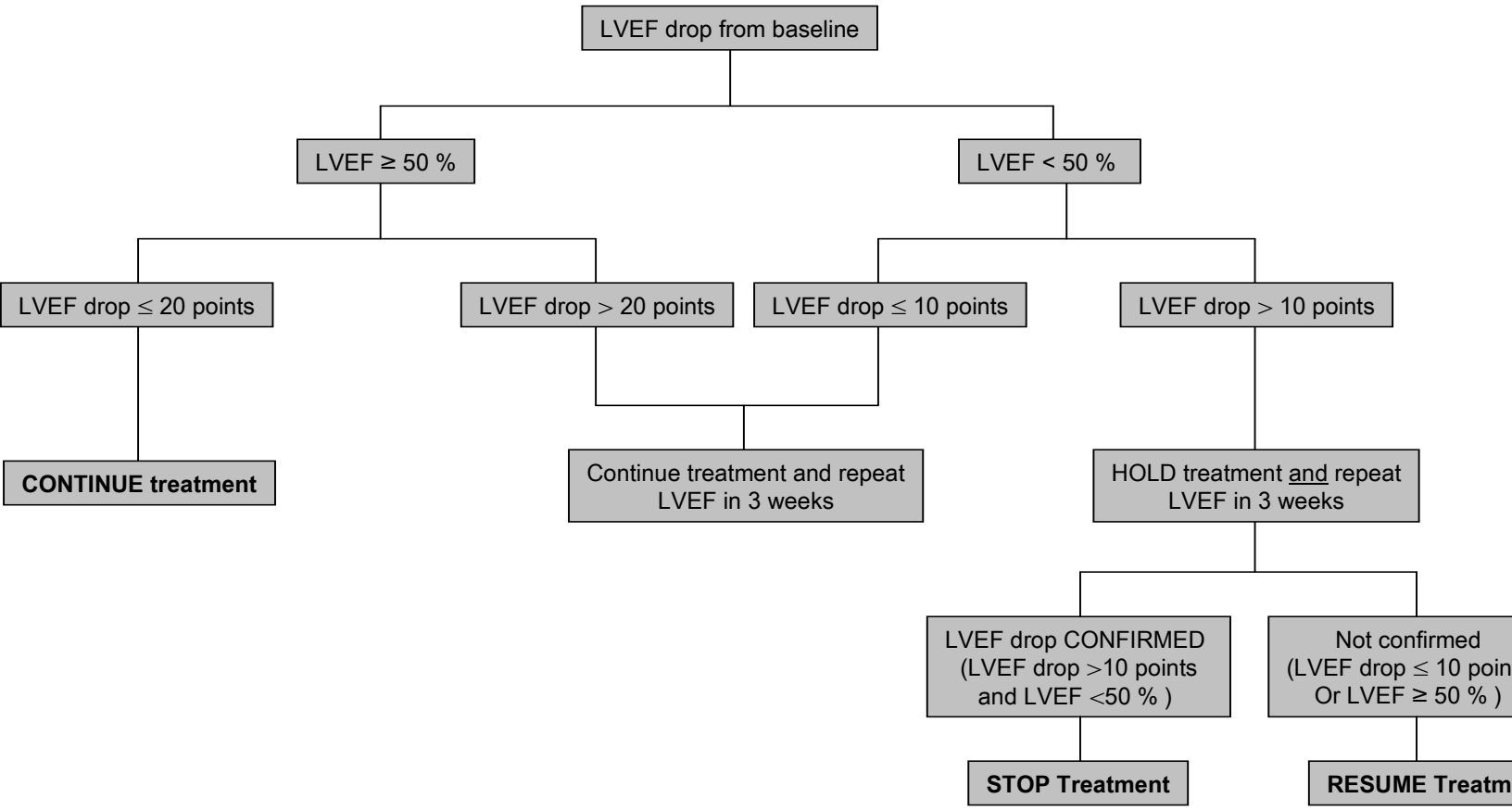
Criteria for Evaluating Cardiac Events:
Asymptomatic cardiac events:

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 investigational product should be temporarily discontinued.
- If the LVEF recovers during the next 3 weeks, after consultation and approval of the medical monitor, the subject may be restarted on investigational product at a reduced dose. For such subjects, monitoring of left ventricular ejection fraction 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 the subject should be withdrawn from investigational product.

Symptomatic cardiac events:
Subjects with an NCI CTCAE grade 3 or 4 LVEF relative decrease must be withdrawn from study medication.

Figure 1 Algorithm for continuation and discontinuation of GW572016 (lapatinib) and/or trastuzumab based on interval LVEF assessments, for patients with NYHA class I or II congestive heart failure



Liver Chemistry Stopping Criteria

Overall, 45% of patients experience hepatic abnormalities on lapatinib and capecitabine. In a phase III trial of lapatinib monotherapy (n = 1573), 8% of patients experienced hepatobiliary events, 3% of those were SAEs. With lapatinib monotherapy, grade 3 hyperbilirubinemia has been reported in 2% of patients and grade 4 elevations in ALT/AST in 2%. Hepatotoxicity has been observed in <1% of subjects. Liver chemistry stopping and follow up criteria have been designed to assure subject safety and evaluate liver event etiology. All subjects who meet liver chemistry criteria requiring permanent discontinuation of investigational product must continue to be followed for the study assessments and procedures as defined in Section X and at the time points indicated in the Time & Events Table in Section X.

If a subject experiences ALT >3 × ULN and total bilirubin >2.0 × ULN (>35% direct; bilirubin fractionation required*), then the following actions must be taken:

immediately and permanently discontinue investigational product;
complete the SAE data collection tool, the liver event CRF, and the liver imaging and/or liver biopsy CRFs, if these tests are performed;
in addition to the liver event follow up assessments defined in Section X.X. below, the following are suggested: specialist or hepatology consultation; anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies; and liver imaging and/or liver biopsy to evaluate liver disease;
promptly report the event to Novartis within 24 hours of learning its occurrence (refer to '**Reporting Serious Adverse Events section'** for guidance on prompt reporting to Novartis);
monitor every week until liver chemistries resolve, stabilize or return to within baseline values;
do not re-challenge with investigational product.

*__NOTE__: bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >2.0 × ULN, then the aforementioned actions must still be performed.

If a subject experiences:

ALT >8 × ULN or
ALT >5 × ULN persisting for ≥2 weeks : retest within 3 days from the first occurrence and then weekly to determine if ALT elevation persists or
ALT >3 × ULN with signs or symptoms of hepatitis or hypersensitivity (the appearance or worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia)

then hold investigational product for 2 weeks, repeat liver chemistry testing in 2 weeks, and then call the Novartis Medical Monitor to discuss the possibility of re-challenging with investigational product.

If the treatment is exhibiting efficacy **and** the subject wants to continue for potential benefit of lapatinib therapy after being informed of the results of liver chemistry testing,

then the investigational product may be re-started at the reduced dose agreed upon by the investigator and the Novartis Medical Monitor. In such cases, Study Visit #8 will not take place if the decision to continue on lapatinib takes place prior to discontinuation of study drug on study. Liver chemistries and aforementioned signs and symptoms should be monitored at a minimum of every 2 weeks until resolution, stabilization, or a return to baseline values, at which point monitoring should be continued per protocol.

If a subject experiences ALT >3 × ULN **but** <5 × ULN **and** total bilirubin ≤2 × ULN, without signs or symptoms of hepatitis or hypersensitivity, **and** who can be monitored weekly, then the following actions should be taken:

continue investigational product;
monitor weekly until liver chemistries resolve, stabilize, or return to within baseline, then monitor liver chemistries as per protocol assessment schedule;
if ALT >3 and < 5 × ULN for > 4 weeks , discontinue the treatment;
if at any time this subject meets any of the aforementioned liver chemistry stopping criteria, then proceed as described above.

Liver Chemistry Follow up Criteria

For all subjects who meet any of the liver chemistry criteria described above, make every attempt to carry out the liver event follow up assessments described below:

Viral hepatitis serology including:
- Hepatitis A IgM antibody;
- Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM);
- Hepatitis C RNA;
- Cytomegalovirus IgM antibody;
- Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing);
- Hepatitis E IgM antibody (if subject resides or has traveled outside USA or Canada in past 3 months);

Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH);
Complete blood count with differential to assess eosinophilia;
Record the appearance or worsening of clinical symptoms of hepatitis, or hypersensitivity, fatigue, decreased appetite, nausea, vomiting, abdominal pain, jaundice, fever, or rash as relevant on an AE report form;
Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins, on the concomitant medications report form;
Record alcohol use on the liver event alcohol intake case report form.

Refer to Appendix 3 for a liver safety algorithm detailing stopping and follow up criteria.

Safety

Add frequency of LFTs to Safety Assessment section and Time & Events Table as applicable:

- During treatment phase: every 4 - 6 weeks (to best fit protocol schedule) for duration of concurrent chemotherapy administration or for first 6 months, then

every 8 – 12 weeks (or more frequently, if clinically indicated) for remaining on treatment period

- During post-treatment phase: continue to monitor any liver chemistry abnormalities noted during treatment or within 30 days after last dose of investigational product until values return to normal or baseline (this language should be in protocols already)

In the Safety Assessment section, make a reference to section containing the Liver Chemistry Stopping and Follow up Criteria.

In the Laboratory Assessment section, mention that bilirubin fractionation is recommended if total bilirubin >2 x ULN when testing is available.

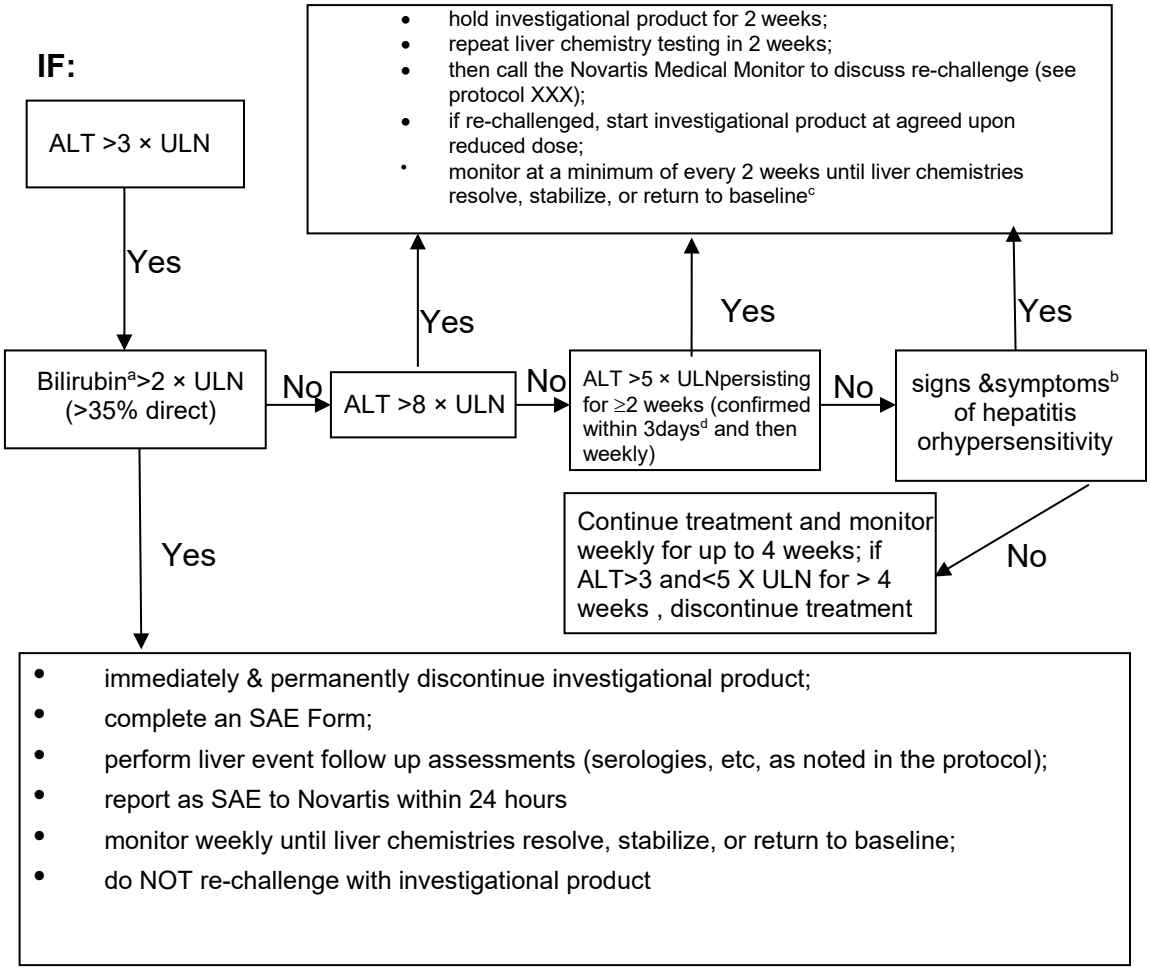
Hepatobiliary events have been seen in subjects taking lapatinib and other tyrosine kinase inhibitors. As a precaution, the following will be reported as an SAE:

- ALT >3 × ULN and total bilirubin >2.0 × ULN (>35% direct; bilirubin fractionation required).

NOTE: bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >2.0 × ULN, then the event should still be reported as an SAE.

Other hepatic events should be documented as an AE or an SAE as appropriate.

Appendix: Liver Chemistry Stopping and Follow up Criteria



a. bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >2.0 × ULN, then the event should still be reported as an SAE and actions taken as described
b. the appearance or worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia
c. once liver chemistries resolve, stabilize, or return to baseline, then continue monitoring per the protocol assessment schedule
d. retest within 3 days from the first occurrence and then weekly to determine if ALT elevation persists

Dermatologic (skin) adverse events

Rash has been reported in 29% of patients on lapatinib monotherapy with significant skin adverse events (Grade 3 or more) resulting in 1-3%.
For NCI-CTCAE v3.0 Grade 4 rash manifested as toxic epidermal necrolysis (i.e. Stevens-Johnson's Syndrome etc) lapatinib must be permanently discontinued.
Subjects with poorly tolerated skin adverse events may be successfully managed by providing a brief (up to 14 days) therapy interruption; the daily dose of lapatinib

should then be reinstated. However, the rash may improve without the need for interrupting therapy with lapatinib. Of note in current studies, many subjects were able to resume lapatinib therapy at the same dose after resolution of rash, and they then had less extensive and/or severe rashes. A variety of agents can be used to manage skin rashes. These include mild-to-moderate strength steroid creams, topical or systemic antibiotics, topical or systemic antihistamines, and occasionally, retinoid creams.

There is no standard, known, or established treatment proven effective for drug-related skin rashes or changes due to lapatinib. If the rash is severe (1-3%) then most commonly, a papular/pustular rash has been observed, which frequently improves even though the same dose of lapatinib therapy is continued uninterrupted. The need for oral or topical antibiotics is a clinical decision of the investigator and should be preceded by a culture of affected areas and, if indicated, a dermatology consultation. Oral retinoids should not be given because of theoretical concerns about negatively affecting the lapatinib mechanism of action. Oral steroids are also strongly discouraged. Other options for treatment of significant rashes may be determined upon consultation with dermatologist.

Gastrointestinal adverse events

Overall 48% of patients on lapatinib monotherapy (n= 1573) experienced diarrhea, with grade 3 diarrhea reported in 21% of subjects and grade 4 diarrhea in 7%. 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 in below.

Nausea, vomiting, or both

Nausea was reported in 18% of patients on lapatinib monotherapy (n=1573). 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 medical monitor); 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.

Management of interstitial lung disease

Pulmonary events occur in approximately 0.2% of patients. If a patient develops symptoms suggestive of interstitial pneumonitis, adult respiratory distress syndrome (ARDS), or non-cardiogenic pulmonary edema, lapatinib or trastuzumab therapy should be interrupted and a thorough evaluation performed. If NCI-CTCAE v3.0 Grade 3 or 4 pneumonitis/fibrosis or pulmonary infiltrate is confirmed (and the relationship to lapatinib and/or trastuzumab cannot be excluded), lapatinib and/or trastuzumab must be permanently discontinued. All incidences of interstitial lung disease/ interstitial pneumonitis regardless of grade must be reported as serious adverse events (SAEs).

Other adverse events

For any other NCI-CTCAE v3.0 Grade 3 or 4 adverse events or any clinically significant, lower-grade adverse event, treatment with lapatinib should be interrupted for a maximum of 14 days until the patient recovers completely or the adverse event reverts to NCI-CTCAE v3.0 Grade 1 or to baseline grade.
If recurrence of adverse event after drug holiday / interruptions is observed a dose reduction by 250mg is recommended. Dose reduction should only be implemented when all supportive care measures have been exhausted without an improvement of patient status. Lapatinib should not be used at doses below 1000mg if given as monotherapy or 750mg in combination with trastuzumab.
In all cases where the subject is withdrawn due to unusual or unusually severe adverse event considered related to lapatinib, the investigator must report the withdrawal as an SAE and on the CRFs.

Table 1 Summary of dose holding/interruptions and dose de-escalation recommendations for GW572016 (lapatinib)in case of lapatinib related adverse events (graded according to NCI-CTCAE v3.0)

Adverse events	Action
Non-hematological, Grade 1 or 2	Continue lapatinib therapy at full dose prescribed (see adverse events management instructions section **Error! Reference source not found.**. Apply maximum supportive care recommendations (see **Error! Reference source not found.**) If prolonged duration of Grade 2 adverse event is affecting quality of life a one-time decrease of dose by 250 mg is allowed.
Non-hematological, Grade 3 or 4	Apply maximum supportive care recommendations (see section **Error! Reference source not found.**) Hold lapatinib therapy until recovery to Grade ≤ 1 (up to 14 days). Refer to events management instructions Section **Error! Reference source not found.**. Grade 3 or 4 left ventricular cardiac dysfunction - please refer to specific section **Error! Reference source not found.** For NCI-CTCAE v3.0 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, a dose reduction by 250mg is recommended. Lapatinib should not be used at doses below 1000mg if given as monotherapy or 750mg in combination with trastuzumab. NOTE: The 750 mg dose should only be used after all supportive measures have been exhausted. (If administered in combination with paclitaxel refer to table 13)
Non-hematological, Grade 3 or 4 and adverse events NOT resolved to Grade ≤ 2 within a maximum of 2 weeks from last planned administration	Action (discontinue or resume lapatinib therapy) in individual cases to be decided by the Executive Committee. Dose reductions by 250mg (up to 2 reductions) will be considered after maximum supportive care recommendations (see **Error! Reference source not found.**) are introduced.

Cardiac* (asymptomatic drop in LVEF or symptomatic congestive heart failure)	Lapatinib therapy to be discontinued permanently in case of symptomatic NYHA class III and IV CHF. Lapatinib therapy to be hold continued or resumed according to Figure 1 for patients with NYHA class I or II CHF.
Hematological adverse events: Absolute neutrophil count (ANC) <1.0 x 10^9/L Platelets <75 x 10^9/L Hemoglobin <9.0 g/dL (after transfusion if needed)	Hold chemotherapy if lapatinib given simultaneously; if the adverse event is related to chemotherapy please refer to appropriate label (until recovery to Grade $\leq$1). If ANC recovers to 1.5 x 10^9/L within 7days, then merely delaying dose is acceptable. If no recovery to ANC of 1.5 x 10^9/L within 7 days then decrease chemotherapy dose by 20%. Dose modifications for thrombocytopenia, at the start of subsequent courses of therapy are the same as recommended for neutropenia above. Next cycle should not begin until the platelet count has recovered to 100 x 10^9/L or more.
	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.

*Severity corresponding to NYHA criteria

Dose Delays and Dose Reductions
Treatment may be delayed, up to 2 weeks, to allow for resolution of toxicity except in the event of NCI CTCAE Grade 3 or 4 left ventricular cardiac dysfunction or NCI CTCAE Grade 3 or 4 interstitial pneumonitis (See Criteria for Evaluating Cardiac and Respiratory Events). The Investigator must consult the Novartis Medical Monitor prior to continuing therapy for any subject requiring a delay of more than 2 weeks for unresolved toxicity, but in general, such subjects should be withdrawn from the study. If treatment is delayed for reasons other than toxicity (i.e., unplanned travel or vacation, or lack of transportation to the site) and the subject has insufficient investigational product available, the subject should resume the usual dosing schedule once drug supply has been made available. However, if the subject has been off therapy for more than 2 weeks, the Investigator must consult the Novartis Medical Monitor prior to continuing therapy.

Dose reduction for drug-related toxicity is permitted; however the Novartis Medical Monitor must be consulted prior to implementing any change in dosing. Only one dose reduction is permitted per subject and subjects should not be rechallenged to a higher dose level. If a NCI CTCAE Grade 3 or 4 drug-related event (other than left ventricular cardiac dysfunction or interstitial pneumonitis) has occurred, the investigator may discuss with the Novartis Medical Monitor whether a reduction of dose is appropriate.

PHARMACEUTICAL INFORMATION

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: None

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

How Supplied: lapatinib is supplied as 250 mg oval, biconvex, orange film-coated tablets with one side plain and the opposite side debossed with FG HLS. The tablets contain 410 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.

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

The film-coat contains: Hydroxypropyl methylcellulose, titanium dioxide, triacetin/glycerol triacetate, and yellow iron oxide.

Storage: The intact bottles should be stored at controlled room temperature (15°C-30°C).

Stability: Shelf life surveillance studies of the intact bottle are on-going. Current data indicates lapatinib is stable for at least 2 years at controlled room temperature (15°C - 30°C).

Route of Administration: Oral on an empty stomach (either 1 hour before or 1 hour after meals).

Kool-Aid Flavored Suspension

Prepare Lemonade or Tropical Punch Kool-Aid as directed on the package. Place 2 or 4 oz of Kool-Aid (room temperature or refrigerated) in a glass container, then add six 250 mg lapatinib tablets to the container. Cover the container, let it stand for 5 minutes, and then stir the mixture intermittently for 10-20 minutes or until the tablets are completely broken up. Stir the container for 5 seconds then administer. Rinse the container with a 2 oz aliquot of water and administer (total of 4-6 oz of liquid is dispensed).

Suspension in Water

Place 4 oz of water in a glass container, then add six 250 mg lapatinib tablets to the container. Cover the container, let it stand for 5 minutes, and then stir the mixture intermittently for 10-20 minutes or until it is fully dispersed. Stir the container for 5 seconds then administer. Rinse the container with a 2 oz aliquot of water and administer (total of 6 oz of liquid is dispensed).

Prohibited Medications

Lapatinib is a substrate for CYP3A4. Inducers and inhibitors of CYP3A4 may alter the metabolism of lapatinib. The following list of CYP3A4 inducers and inhibitors are prohibited from screening through discontinuation from study.

Drug Class	Specific Agents	Wash-out[1]
CYP3A4 Inducers		
rifamycin antibiotics	rifampicin, rifabutin, rifapentine	2 weeks
anticonvulsants	phenytoin, carbamezepine, barbiturates (e.g., phenobarbital)	
antiretrovirals	efavirenz, nevirapine, tipranivir, etravirine	
glucocortical steroids (oral only)	cortisone (>50 mg), hydrocortisone (>40 mg), prednisone or prednisolone (>10 mg), methylprednisolone or triamcinolone (>8 mg), betamethasone or dexamethasone (>1.5 mg)[2]	
other	St. John's Wort, modafinil	
CYP3A4 Inhibitors		
antibiotics	clarithromycin, erythromycin, troleandomycin, flucloxacillin	1 week
antifungals	itraconazole, ketoconazole, fluconazole (>150 mg daily), voriconazole	
antiretrovirals	delaviridine, nelfinavir, amprenavir, ritonavir, indinavir, saquinavir, lopinavir, atazanavir	
calcium channel blockers	verapamil, diltiazem	
antidepressants	nefazodone, fluvoxamine	

gastrointestinal agents[3]	cimetidine	
fruit juices	grapefruit, star fruit, and papaw	
other	amiodarone	6 months
Miscellaneous		
antacids	Mylanta, Maalox, Tums, Rennies	1 hour before and after dosing
herbal supplements[4]	ginkgo biloba, kava, grape seed, valerian, ginseng, echinacea, evening primrose oil	2 weeks

1. Time period between last dose of listed drug and first dose of lapatinib, required to avoid drug-drug interaction potential for toxicity (inhibitors) or loss of efficacy (inducers) that could make the patient unevaluable. Clinically appropriate substitution of drugs not on the list is recommended.

2. A standard 3-5 day course of dexamethasone at a dose following the institutions standard of care for the prevention and/or treatment of platinum-induced nausea and vomiting is allowed. Glucocortical steroid oral dose equivalents (in parentheses) to dexamethasone 1.5 mg (or less) given daily are allowed. Intravenous dosing should be considered if clinically appropriate.

3. Emetogenic chemotherapy may require 3-4 daily doses of aprepitant. CYP3A4 inhibition by oral (not IV) aprepitant may require a concurrent dose reduction of 1-2 lapatinib tablets.

4. This list is not all-inclusive; therefore, for herbal supplements not listed, please contact a Novartis Medical Monitor or Clinical Scientist.

NOTE: If future changes are made to the list of prohibited medications, formal documentation will be created and stored with the study file. Any changes will be communicated to the investigative sites in the form of a letter.

Agent Accountability

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 Novartis.

REGULATORY AND REPORTING REQUIREMENTS

It is the responsibility of the investigator to document all adverse events which occur during the investigation. An adverse event is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of the medicinal product, whether or not considered related to the medicinal product. *An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.* Anticipated day-to-day fluctuations of the disease under study that do not represent a clinically significant exacerbation or worsening need not be considered an adverse event.

All adverse events occurring from the first dose of investigational product until five days after the last dose must be recorded **REGARDLESS OF WHETHER OR NOT THEY ARE CONSIDERED DRUG RELATED.** In addition, any SAEs which occur as a result of protocol specific diagnostic procedures or interventions must also be reported.

It is the responsibility of the IND holder to comply with IND safety reporting as set forth in the Code of Federal Regulations, Section 312.32.

Assessment of Causality

Every effort should be made by the investigator to explain each adverse event and assess its relationship, if any, to study drug treatment. Causality should be assessed using the following categories: no (not related), or yes (reasonable possibility).

The degree of certainty with which an adverse experience is attributed to drug treatment (or alternative causes, e.g. natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the experience can be understood in terms of the following:

- Known pharmacology of the drug

- Reaction of similar nature being previously observed with this drug or class of drug

- The event having often been reported in literature for similar drugs as drug related (e.g. skin rashes, blood dyscrasia)

- The event being related by time to drug administration terminating with drug withdrawal (dechallenge) or reproduced on rechallenge.

The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE form. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Following-up of Adverse Events

Investigators should follow-up subjects with adverse events until the event has subsided (disappeared) or until the condition has stabilized.

Definition of Serious Adverse Events:

A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)

- elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
- treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- social reasons and respite care in the absence of any deterioration in the patient's general condition

- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

Abnormal laboratory findings (e.g., clinical chemistry, hematology) or other abnormal assessments (e.g., x-rays, scans, vital signs, etc.) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE.

Life threatening definition:

An adverse event is life threatening if the patient was at immediate risk of death from the event as it occurred (i.e. it does not include a reaction that if it had occurred in a more serious form might have caused death). For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug-induced hepatitis could be fatal.

Disability/incapacitating definition:

An adverse experience is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the patient's ability to carry out normal life functions.

Hospitalization definition:
Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

Additional SAE definitions
- All Grade 4 laboratory abnormalities
- Cardiovascular events have been seen in subjects taking other compounds that inhibit ErbB2 when used in combination with or following anthracyclines and interstitial pneumonitis has been reported in subjects taking compounds that inhibit ErbB1. As a precaution, the following will be reported as a SAE:

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 left ventricular cardiac ejection fraction relative to baseline, which is below the institution's lower limit of normal. Refer to NCI CTCAE grading of left ventricular cardiac function.

Hepatobiliary events have been seen in subjects taking lapatinib and other tyrosine kinase inhibitors. As a precaution, the following will be reported as an SAE:

- ALT >3 × ULN and total bilirubin >2.0 × ULN (>35% direct; bilirubin fractionation required).

NOTE: bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >2.0 × ULN, then the event should still be reported as an SAE.

Other hepatic events should be documented as an AE or an SAE as appropriate.

SAEs, pregnancies, and liver function abnormalities meeting pre-defined stopping criteria will be reported promptly to Novartis as described in the following table once the investigator determines that the event meets the protocol definition for that event.

	Initial Reports		Follow-up Information on a Previous Report	
Type of Event	Time Frame	Documents	Time Frame	Documents
All SAEs	24 hours	"SAE" data collection tool	24 hours	Updated "SAE" data collection tool
Pregnancy	2 Weeks	Pregnancy Notification Form	2 Weeks	Pregnancy Follow up Form
Liver chemistry abnormalities:				
ALT >3 × ULN **and** bilirubin[a]>2 × ULN (35% direct)	24 hours	CRF	24 hours	CRF

a. bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >2.0 × ULN, then the event should still be promptly reported as defined.

Reporting Serious Adverse Events

Any serious adverse events which occur during the clinical study or within 30 days of receiving the last dose of study medication, whether or not related to the study drug, must be reported by the investigator. In addition, any SAEs which occur as a result of protocol specific diagnostic procedures or interventions must also be reported.

The principal investigator has the obligation to report all serious adverse events to the FDA, IRB, and Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E) ***(For patients taking Lapatinib / Novartis drugs)***.

All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form).

To ensure patient safety, every SAE, regardless of suspected causality, occurring

- after the patient has provided informed consent and until atleast 30 days after the patient has stopped study treatment/participation
- after protocol-specified procedures begin (e.g., placebo run-in, washout period, double-blind treatment, etc.) and 30 days after the patient has stopped study treatment

- after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient (e.g., treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication) and until 30 days after the patient has stopped study treatment

must be reported to Novartis within 24 hours of learning of its occurrence Information about all SAEs is collected and recorded on a Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and **send the completed, signed form by fax to (fax: 877-778-9739) within 24 hours to the oncology Novartis DS&E department with the provided FAX cover sheets**.

. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any SAEs experienced after this 30 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

The original copy of the SAE Report and the fax confirmation sheet must be kept within the Trial Master File at the study site.

Follow-up information is sent to the same fax number as the original SAE Report Form was sent, using a new fax cover sheet, stating that this is a follow-up to the previously reported SAE, and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Lapatinib Investigator Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study drug, a DS&E associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence.

Lack of Efficacy

"Lack of efficacy" *per se* will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the AE or SAE definition (including clarifications).

All serious adverse events, in addition to being reported to the FDA by the investigator, must be reported by facsimile within 24 hours to Novartis Oncology.

The SAE report should comprise a full written summary, detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports should be included. Follow-up information should be forwarded to Novartis within 24 hours.

SAEs brought to the attention of the investigator at any time after cessation of lapatinib and considered by the investigator to be related or possibly related to lapatinib must be reported to Novartis if and when they occur. Additionally, in order to fulfill international reporting obligations, SAEs that are related to study participation (e.g., procedures, invasive tests, change from existing therapy) or are related
to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until he/she is discharged.

Pregnancy

Patients who become pregnant during the study should discontinue the study immediately.

Patients should be instructed to notify the investigator if it is determined after completion of the study that they become pregnant either during the treatment phase of the study or within five days after the treatment period.

Whenever possible a pregnancy should be followed to term, any premature termination reported, and the status of the mother and child should be reported to GlaxoSmithKline after delivery.

Section 9.0. Statistics

Sample size calculation for prolactinomas: Sample size was computed using proportions as standard deviation for tumor reduction on the study drug was unknown. While for the data analysis we will use quantitative analysis using the Hotelling's T2, using binary data for power calculations would be inefficient as this would require a larger sample size. We could not use the quantitative analysis for power calculations, however, as we do not know the variance.
The primary outcome is a reduction in maximal size of resistant tumors. Stabilization is defined as <20% reduction in any dimension on MRI by RECIST criteria. MRIs will be performed at baseline and at 3 and 6 months.
The null hypothesis is H_0: no reduction $P<0.20$ vs. the alternative hypothesis H_a: $P>0.40$. This will give an effect size of 0.20 to detect. Exact confidence interval and significance test is obtained from the binomial distribution. The reduction is for resistant prolactinomas (Di Sarno, 2001; Delgrange, 2009). Power is >80%, using one tailed

hypothesis, alpha of 0.10. This gives a target sample size of 24 subjects to achieve this power.

Sample size: use one-sided exact test. H_0: $p = p_0$; H_A: $p > p_0$.

Power	alpha	P_0: null proportion	P_1: true proportion	N
80%	0.05	20%	30%	116
80%	0.10	20%	30%	88
80%	0.05	20%	35%	56
80%	0.10	20%	35%	44
80%	0.05	20%	40%	35
80%	0.10	20%	40%	24

Secondary MRI studies will deal with various aspects and location by MRI of these tumors. Secondary PRL statistical studies will also be carried out under secondary outcomes.

Tumor maximal size will be obtained at baseline, 3 months, 6 months after initiation of treatment. The primary hypothesis is the change from baseline to 6 months. The analysis using maximal resistant tumor size will be carried out for the specified times given above. Initially this will be by descriptive analytic and then by inferential analysis using the test for change between baseline and 6 months and change between baseline and 3 months using rank test. We will also use Hotelling's T^2 to combine both pre-post changes.

This study is in the clinical trials paradigm essentially a Phase IIa trial and thus use of alpha type 1 error is at 10%. This is sanctioned by the biostatistics section of NCI.[40] We will also analyze the secondary endpoints of Aim 1 according to the above models. Concepts behind analysis for quantitative and binary are the same though the methods are different.

Our overall target sample size is 30 subjects which includes nonfunctioning adenomas and prolactinomas, 15 to be recruited from Cedars Sinai Medical Center and 15 from Johns Hopkins Hospital and Massachusetts General Hospital. We aim to recruit 24 subjects with prolactinomas and 6 nonfunctioning adenomas.

Due to the rarity of pituitary tumors in the general population and even more rare are aggressive pituitary tumors such as we propose to study, it is difficult to do a randomized placebo controlled trial with sufficient sample size numbers to achieve adequate power. By using the subjects as their own controls, we alleviate the issues with recruitment. This is a safety and activity trial to look for a sufficient effect of the drug on tumor growth before proceeding to a larger, randomized placebo controlled trial.

Section 10.0. Quality Control and Quality Assurance

Only samples and data collected with appropriate consent will be used for the proposed analysis. Specimens and data collected under the proof of concept clinical trial of lapatinib will be coded in such a way so that other members of the research team will be unable to ascertain the identity of specific patients. All of our data will be

maintained confidentially. Data will be kept in locked file cabinets and in password protected files on computer systems.

Section 11.0 Ethical considerations relating to the trial

Subjects will be recruited from the Pituitary Center. They will be patients followed clinically. Patients will be approached regarding participation in the study and given consent forms to review at home. They will be reassured that their clinical care will not be affected if they choose not to participate. Patients will be given opportunities to ask questions and review the consent process. Once they consent and enroll in the study, their data will be deidentified for the duration of the study. All patients entering the trial will be given the study drug. There is no placebo arm. The risks of the study are related to the side effects of the study drug and patients will be monitored for this and informed on the effects. The benefits of the study are stabilization of tumor growth.
There are potential conflicts of interest between the patient's need for surgical referral and participation in the research study. However, by excluding patients who would meet definite criteria for surgery, we will minimize the conflict of interest.

Section 12.0. Data Handling and Recordkeeping

Data will be maintained in separate research charts in a locked cabinet in a locked office.
The data will be entered as well into an electronic database on a password protected computer. Only study staff and the investigators will have access to these records.

Section 13.0. Financing and Insurance

Funding for this study will be through institutional funds. Patients will only be charged for the initial MRI and visual field test, as well as hormonal tests during the study period.

Section 14.0. Publication Policy

We intend to submit our research findings to relevant peer-reviewed journals for publication. We will submit data for presentation at international meetings. We will adhere to the NIH Grants Policy Statement on Sharing of Biomedical Research Resources, including the "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice" (64FR 72090, December 23, 1999; and described at http://ott.od.nih.gov/NewPages/RTguide/final.html.