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Permalink

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Journal

Gynecologic Oncology, 127(2)

ISSN

0090-8258

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Publication Date

2012-11-01

DOI

10.1016/j.ygyno.2012.07.127

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Lapatinib and potential prognostic value of EGFR mutations in a Gynecologic Oncology Group phase II trial of persistent or recurrent endometrial cancer^{☆,☆☆}

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HIGHLIGHTS

- ▶ This study, GOG 229D, was one of the first to employ molecular therapy for advanced and recurrent endometrial cancer.
- ▶ Lapatinib, an inhibitor of EGFR and HER-2, had limited clinical activity in unselected patients.
- ▶ We report a newly identified EGFR mutation which correlated with response in one patient.

ARTICLE INFO

Article history:

Received 28 June 2012

Accepted 31 July 2012

Available online 7 August 2012

Keywords:

Endometrial cancer

Epidermal growth factor receptor

Mutation

Lapatinib

Tyrosine kinase inhibitor

ABSTRACT

Objective. A phase II trial was performed to evaluate the efficacy and safety of the tyrosine kinase inhibitor of epidermal growth factor receptor (EGFR) and HER2, lapatinib, and to explore EGFR, HER2 (EGFR2), phosphorylated ERK MAP kinase (pERK), and Ki67 expression, as well as EGFR mutations in persistent/recurrent endometrial cancer (EC).

Methods. Women with histologically-confirmed, measurable, persistent/recurrent EC following one or two prior regimens were eligible and treated with 1500 mg oral lapatinib daily until progression or severe toxicity. A 2-stage group sequential design was used to evaluate the regimen with 6 month PFS as the primary endpoint. The trial had a 10% type I error rate with 90% power. EGFR, HER2, pERK, and Ki67 were evaluated by immunohistochemistry (IHC) from hysterectomy specimens, pre-treatment biopsies, and post-treatment biopsies (when available). Exons 18–21 of EGFR were sequenced.

Results. Three patients of 30 evaluable had PFS \geq 6 months, one had a partial response, seven had stable disease, 21 had progressive disease and one was indeterminate. Three mutations in EGFR were identified. Two of these, L688F and K754E, were not associated with response or PFS. However, a newly identified mutation in exon 18, E690K, occurred in the patient with a partial response and progression-free survival extending past six months.

Conclusion. While lapatinib has limited activity in unselected cases, the identification of a previously unreported mutation in EGFR (E690K) with a response suggests that lapatinib may be beneficial in some cases of EC.

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[☆] This study was supported by National Cancer Institute grants to the Gynecologic Oncology Group (GOG) Administrative Office and the GOG Core Laboratory for Receptors and Targets (CA 27469), the GOG Tissue Bank (CA 11479), the GOG Statistical and Data Center (CA 37517), R01CA99908 (KL) and the Cory Beach Family Fund for Endometrial Cancer Research.

^{☆☆} The following GOG member institutions participated in this protocol: Abington Memorial Hospital, University of California at Los Angeles, University of Washington, University of North Carolina School of Medicine, University of Iowa Hospitals and Clinics, University of California Medical Center at Irvine, SUNY Downstate Medical Center, University of New Mexico, The Cleveland Clinic Foundation, Washington University School of Medicine, University of Oklahoma, Tacoma General Hospital, and Ellis Fischel Cancer Center.

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Introduction

Advanced endometrial cancer is a lethal disease for which effective therapies are lacking. More than 8000 women in the U.S. are estimated to have died of endometrial cancer in 2010. According to data from the American Cancer Society Facts and Figures, 2011, relative five-year survival has not improved and is, in fact, worse today than it was in 1975 (83% versus 88%). In an attempt to prolong progression-free survival (PFS) in this cohort, the Gynecologic Oncology Group (GOG) runs a phase II queue for molecular therapies.

Growth factors and their receptors are known to play critical roles in endometrial cell growth and carcinogenesis [1]. Many of these receptors possess intrinsic tyrosine kinase activity that is activated upon binding of the receptor with its ligand. The epidermal growth factor (EGF) is a potent mitogen for several human epithelial cell types including endometrium and has been implicated in cancer development. The EGF receptor (EGFR) has been shown to be highly expressed in endometrial cancer [2]. Human epidermal growth factor receptor 2 (HER2), otherwise known as EGFR2, is another member of the EGFR family which has been associated with poor outcome when amplified [3,4]. HER2 binds to EGFR to compose one of the functional tyrosine kinase receptor dimers activated by EGF and other EGF-like ligands such as amphiregulin [5].

Somatic EGFR mutations in the tyrosine kinase domain (exons 18–21) identify lung tumors dependent on this pathway for growth and proliferation and appear to sensitize tumors to the effects of adenosine triphosphate (ATP)-mimetic, small molecule inhibitors. Several reports suggest that EGFR mutations confer survival benefit independent of treatment and with tyrosine kinase inhibitor therapy [6–8].

Lapatinib acts as a dual inhibitor of both EGFR and HER2 tyrosine kinase activity [5]. As a member of the 4-anilinoquinazoline class of kinase inhibitors, lapatinib is thought to bind to and block the ATP binding sites of the receptor dimer, resulting in inhibition of auto-phosphorylation and subsequent proliferative signaling [9]. This phase II clinical trial tested the hypothesis that lapatinib has clinical activity against advanced or recurrent endometrial cancer which correlates with the biological characteristics of the tumor such as immunohistochemical analysis of target signaling molecules and EGFR mutations.

Methods

Study overview

The study reported herein is a phase II open-label trial evaluating the efficacy and safety of lapatinib (GW572016, Tykerb/Tyverb, GlaxoSmithKline, Research Triangle Park, NC), an epidermal growth factor receptor (EGFR) and HER2 dual tyrosine kinase inhibitor, in 30 evaluable patients with endometrial carcinoma who had persistent or recurrent disease following front-line chemotherapy and possibly one salvage regimen. The translational science component of this study included correlating clinical response with the biological characteristics of the tumor including EGFR, HER2, phosphorylated ERK MAP kinase (pERK), and Ki67 immunostaining, as well as EGFR mutations.

Eligibility

Eligible patients had histological diagnosis of recurrent or persistent endometrial carcinoma as established by central review by the GOG Pathology Committee. Patients were required to be 18 years of age or older and have measurable disease as defined by Response Evaluation Criteria in Solid Tumors (RECIST) [10], a GOG performance status of 0–2, and adequate bone marrow (absolute neutrophil count $\geq 1500/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$), renal (serum creatinine $\leq 1.5 \times$ the upper limit of normal), and hepatic function (total bilirubin $\leq 1.5 \times$ the upper limit of normal, transaminases and alkaline phosphatase $\leq 2 \times$ the upper limit of normal). Eligible patients were permitted to

have up to two prior cytotoxic regimens. Patients with prior treatment for recurrent disease with a non-cytotoxic agent, prior radiation to more than 25% of marrow bearing areas, therapeutic warfarin treatment, inability to take oral medications, or concurrent use of CYP3A4 inducers or inhibitors were ineligible.

Patients provided written informed consent consistent with federal, state, and local institutional requirements and authorization permitting release of personal health information. The protocol was approved by the Institutional Review Board at each participating GOG institution and performed in accordance with assurances filed with and approved by the Department of Health and Human Services.

Treatment plan and dose modifications

Lapatinib was started at a fixed dose of 1500 mg once a day until progression of disease or adverse effects prohibited further therapy. A cycle equaled 28 days. Lapatinib was supplied by the Cancer Treatment Evaluation Program of the National Cancer Institute (NCI). Toxicity was graded using the NCI Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0). Patients who experienced neutropenic fever, grade 4 neutropenia lasting more than seven days, or grade 4 thrombocytopenia had treatment delayed until toxicity resolved to grade 1 or less; subsequently their dose was reduced one dose level to 1000 mg daily. If the same toxicity recurred, then the next lower dose level was 750 mg daily, with no further reductions permitted. Use of erythroid growth factor was permitted after the hemoglobin dropped below 10 g/dL.

Response assessment

Patients were evaluated clinically every four weeks and radiologically every eight weeks. The same evaluation modality was used throughout for each patient on study. Response criteria used were as defined by RECIST. PFS was the period of time from study entry until death, disease progression, or date of last contact (whichever occurred first). Overall survival (OS) was the period of time from entry until death or last contact.

Immunohistochemistry

Archived formalin-fixed, paraffin-embedded (FFPE) primary tumor tissue from the initial hysterectomy and recurrent or persistent endometrial tumor tissue obtained by a core biopsy or fine needle aspiration were collected. The original hysterectomy primary tumor was available for 27 cases, and a pre-treatment biopsy of recurrent tumor was available for 24 cases. A post-treatment biopsy, performed after 3 cycles of therapy, was optional in this protocol, and was rarely performed.

Unstained sections of primary tumor and biopsies were shipped from the GOG Tissue Bank to the GOG Core Laboratory for Receptors and Targets for immunohistochemical (IHC) analysis as previously described [11]. EGFR, pERK, Ki67, and HER2 were analyzed by IHC using sections of FFPE primary tumor and pre-treatment biopsy tissue. EGFR and HER2 were chosen for analysis because they are the targets of lapatinib. pERK was used as a marker to assess the activity of the MAP kinase signaling pathway downstream of EGFR–HER2, and Ki67 was used as a marker for cellular DNA synthesis. Positive and negative controls were included in each run. For the negative control sections, the primary antibody was substituted with immune serum. HER2, EGFR, and Ki67 were performed on the Ventana Autostainer (Tucson, AZ) using standard protocols and antibodies supplied by Ventana. The pERK staining was performed by hand using Cell Signaling antibody catalog #4376 at a 1:25 dilution and an antigen retrieval time of 5 min. Intensities were scored as 0, 1+, 2+, or 3+ compared to strongly staining breast cancer tissue used as a positive control and staining in the absence of the primary antibody as a negative control. Intensities were multiplied by the percent of cells staining to derive a modified H score. An experienced pathologist (EF) read the slides in a blinded fashion.

Analysis of EGFR mutations

Genomic DNA was extracted from FFPE tumor tissue using a Trimgen DNA purification kit (Trimgen Corp, Sparks, MD) according to the kit instructions. EGFR exons 18–21 were amplified by polymerase chain reaction (PCR) as published previously [12]. PCR amplicons were cleaned with QIAquick® PCR Purification Kit (Qiagen Inc., Valencia, CA) and were subjected to direct sequencing using the same PCR primers and the ABI BigDye Terminator kit v1.1 (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Sequence variations were determined by using Sequencher software 4.7 (Gene Codes Corporation, Ann Arbor, MI) and compared with GenBank genomic sequences. All of the sequence variations were confirmed by multiple, independent PCR amplifications and repeated sequencing reactions. Mutational analyses were performed without knowledge of clinical outcome including tumor response.

Statistical methods

The primary objectives of the study were to assess the efficacy and toxicity of lapatinib. The primary endpoint to evaluate efficacy was six month PFS. Patients who were six months progression-free were classified as positive outcomes. All others were considered treatment failures even if their follow-up time was less than six months.

The clinical trial utilized a two-stage flexible group sequential design [13,14]. Briefly, 25 patients were targeted for the first stage of accrual but allowed to deviate for administrative flexibility. If more than four of 30 patients were six months progression-free, then a cumulative of 56 patients was targeted, requiring more than 11 patients with six month PFS before considering the drug for further evaluation in a phase III study [14]. If the true probability of having six month PFS is 15%, the probability of designating the treatment as active was 8.7%, and the average probability of early termination was 52%. If the true probability of having six month PFS was 30%, then the probability of classifying the treatment as active was 90% with a 3% probability of early termination.

Secondary clinical objectives included the characterization of the distribution of PFS and OS. The proportion of patients responding (partial and complete) was also calculated with 90% confidence intervals (CI) assuming a binomial distribution.

Evaluation of translational research was conducted with biomarker data (IHC and mutation analyses) against patient demographics and clinical outcome including six month PFS, tumor response, PFS, and OS. IHC data was expressed in three forms for each biomarker (intensity, percent staining positive, and a product of the two called a modified "H score"). These values were further dichotomized into high and low values (greater or less than the observed median value, respectively). Depending on the nature of the data, associations were examined with Kendall's or Spearman's correlation, Fisher's Exact Test, or an Exact Chi-Square Test [13–16]. Kaplan–Meier plots and Cox proportional hazard models were used to assess the associations between the dichotomized biomarkers with PFS and OS [17,18]. Associations were considered suggestive if any test had a p-value <0.05, which were used to generate hypotheses in future studies. The statistical power of these tests was small, so negative results should not be considered conclusive.

Results

Patient characteristics and tumor subtypes

Thirty-one patients were entered onto the trial. Patient demographics, clinical, tumor, and prior treatment characteristics are summarized in Table 1. Of these, one individual was never treated, leaving 30 evaluable patients. The majority of patients (n=21) entering the trial were 60 or older and white (n=24). The tumor subtypes represented a spectrum of endometrial cancers with 16 of the tumors being endometrioid adenocarcinomas. See Table 1 for further details.

Table 1
Clinical characteristics and tumor type.

Characteristic	Category	No. of cases	% of cases
Age	30–39	2	6.7
	40–49	4	13.3
	50–59	3	10.0
	60–69	13	43.3
	70–79	8	26.7
Race	Asian	1	3.3
	African American	3	10.0
	American Indian	2	6.7
	White	24	80.0
Performance status	0	19	63.3
	1	11	36.7
Site of disease	Corpus	29	96.7
	Trophoblastic	1	3.3
Cell type	Adenocarcinoma, Unsp.	2	6.7
	Clear cell carcinoma	3	10.0
	Endometrioid adenocarcinoma	16	53.3
	Mixed epithelial carcinoma	2	6.7
	Serous adenocarcinoma	7	23.3
Grade	1	3	10.0
	2	6	20.0
	3	14	46.7
	Not graded	7	23.3
Prior chemotherapy	1 prior regimen	16	53.3
	2 prior regimens	14	46.7
Prior radiation	No	19	63.3
	Yes	11	36.7
Prior surgery	No	1	3.3
	Yes	29	96.7

Clinical outcomes

Three patients had six months PFS (10%; 90% CI 2.3%–23.9%). One patient (3.3%; 90% CI 0.2%–14.9%) had a partial response, seven patients (23.3%) had stable disease, 21 patients (70%) had increasing disease, and one was indeterminate for response evaluation. Fig. 1 provides the Kaplan–Meier curve for PFS and OS. Median PFS and OS were 1.82 and 7.33 months, respectively.

Adverse events

There were 97 cycles of therapy administered to 30 patients. The median number of cycles was two (range 1–35). In general, lapatinib was well-tolerated; adverse events are provided in Table 2. Two patients experienced a grade 4 AE, one for anemia and another for a laboratory/metabolic finding (elevated serum creatinine). The most

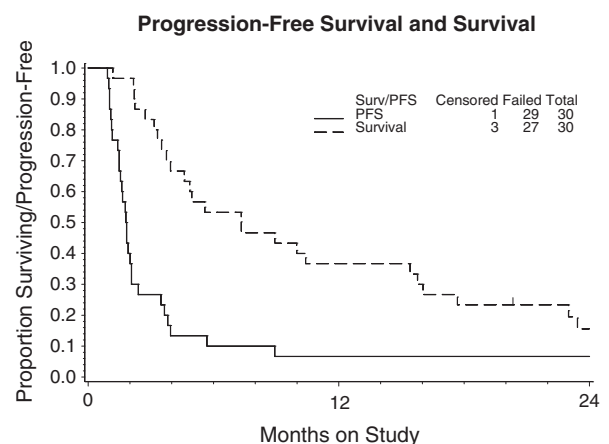


Fig. 1. Kaplan–Meier Curve for patients in GOG 229D. The solid line represents cases with progression-free survival, and the dashed line represents surviving cases.

Table 2
Adverse events.

Grade						
Adverse effect	0	1	2	3	4	Total
Leukopenia	29	1	0	0	0	30
Thrombocytopenia	28	2	0	0	0	30
Anemia	11	8	10	0	1	30
Cardiovascular	28	1	0	1	0	30
Constitutional	13	10	7	0	0	30
Dermatologic	22	6	1	1	0	30
Gastrointestinal	5	12	7	6	0	30
Genitourinary/renal	29	0	0	1	0	30
Hemorrhage	29	0	0	1	0	30
Lymphatics	29	0	1	0	0	30
Musculoskeletal	27	2	1	0	0	30
Metabolic	20	5	2	2	1	30
Neuropathy	29	1	0	0	0	30
Other neurologic	27	3	0	0	0	30
Ocular	26	4	0	0	0	30
Pain	25	4	1	0	0	30
Pulmonary	27	1	1	1	0	30

common side effect was gastrointestinal, followed by anemia and constitutional symptoms. Cardiovascular side effects, a concern when using EGFR blocking agents, were rare; however, one patient experienced a grade 3 AE (left ventricular systolic dysfunction).

Immunohistochemistry

Twenty-seven (90%) women had tissue for evaluation and were included in the analysis. The data were evaluated separately in those patients who progressed versus those who did not and are summarized in Table 3. On the initial hysterectomy specimens, EGFR was expressed in 62% (n = 15) of 24 primary tumors analyzed and had a positive correlation with pERK expression (Spearman correlation 0.49; 95% CI 0.11–0.89). In comparison to the frequent finding of positive staining for EGFR and pERK (Fig. 2), HER2 expression was rare, with only 8% (n = 2) of the tumors scored as positive. EGFR was expressed in 71% (n = 12) of 17 pre-treatment recurrent biopsies analyzed; HER2 expression remained rare in the pre-treatment biopsies, with 12% (n = 3) expressing this molecule in these recurrent lesions. Ki67 staining was common in primary and recurrent tumors, and there was a suggested inverse association between intensity of Ki67 in primary tumor samples and GOG performance status. There was also a suggested association between intensity of Ki67 in recurrent pre-treatment biopsies and response by the permutation test (Table 3).

In summary, EGFR, pERK and Ki67, but not HER2, were present in a significant number of tumor samples from hysterectomy (primary) and pre-treatment (recurrent) biopsies. Pre-treatment recurrent EGFR expression was higher in patients with no disease progression, suggesting a correlation between the expression of a drug target, EGFR, and outcome.

Mutation analysis

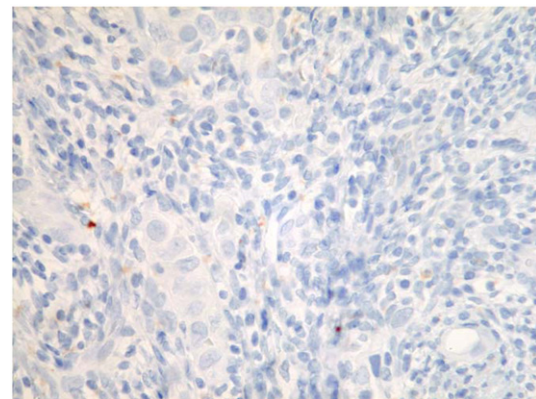
DNA was isolated from sections of FFPE pre-treatment primary tumor collected from 30 women, and the EGFR tyrosine kinase domain exons were sequenced. Twenty-eight (93%) women were included in the analysis. Three mutations were observed – two in exon 18 (L688F, E690K) and one in exon 19 (K754E) (Fig. 3). Each mutation was observed in separate patients. There were multiple silent alterations (presumed polymorphisms) observed and none were associated with clinical characteristics. However, the patient with the E690K mutation had a clinical response, which was suggestive for an association (95% one-sided CI for the odds ratio was 1.42 – infinity).

Table 3
Immunohistochemistry modified H scores segregated by disease progression.

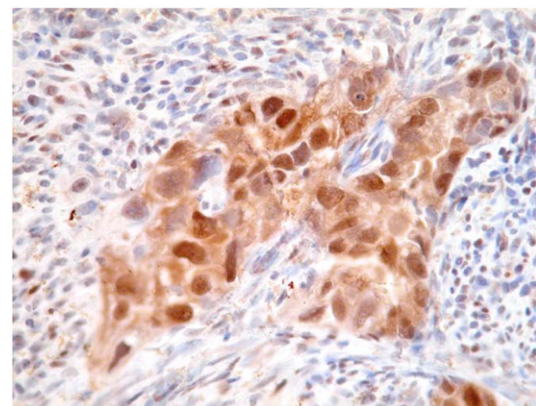
No disease progression		Variable	n	Lower quartile	Median	Upper quartile
Primary tumor	EGFR	8	0.00	70.00	145.00	
	pERK nucleus	8	0.00	0.00	10.00	
	pERK cytoplasm	8	0.00	0.00	0.00	
	Ki67	8	50.00	125.00	165.00	
Recurrent tumor	EGFR ^a	6	15.00	110.00	240.00	
	pERK nucleus	7	0.00	30.00	80.00	
	pERK cytoplasm	7	0.00	0.00	0.00	
	Ki67 ^b	7	90.00	120.00	210.00	
Disease progression		Variable	n	Lower quartile	Median	Upper quartile
Primary tumor	EGFR	16	0.00	15.00	75.00	
	pERK nucleus	18	0.00	2.50	40.00	
	pERK cytoplasm	18	0.00	0.00	0.00	
	Ki67	19	20.00	80.00	150.00	
Recurrent tumor	EGFR ^a	11	0.00	5.00	40.00	
	pERK nucleus	15	0.00	10.00	100.00	
	pERK cytoplasm	15	0.00	0.00	0.00	
	Ki67 ^b	17	40.00	90.00	120.00	

^aHigher values of EGFR on recurrent, pre-treatment biopsies were associated with no progression.

^bHigher values of Ki67 on recurrent, pre-treatment biopsies were associated with no progression.



pERK negative control



pERK positive control

Fig. 2. pERK immunostaining controls. Note the presence of pERK in both the nuclear and cytoplasmic cellular compartments in the positive control.

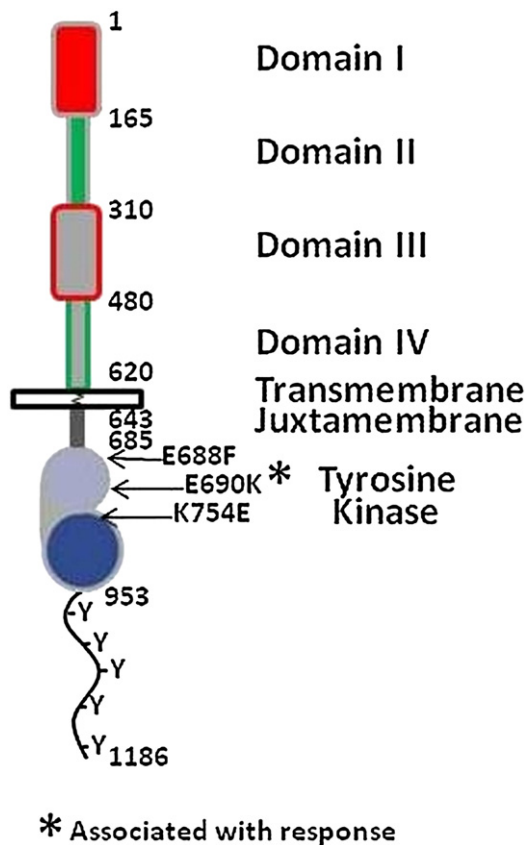


Fig. 3. Epidermal growth factor receptor structure and identified mutations. The mutations identified in this study are shown within the tyrosine kinase domain. One mutation, E690K, was found in the patient who experienced a partial response and prolonged progression-free survival.

Discussion

Lapatinib (GW572016, Tykerb/Tyverb) is the first dual inhibitor of EGFR and HER2 in clinical use. This agent inhibits the intracellular phosphorylation of the EGFR–HER2 complex and prevents signaling downstream. In comparison to trastuzumab (Herceptin), the monoclonal antibody against HER2, lapatinib has several advantages which supported its choice for GOG study. Lapatinib is a less expensive oral agent which inhibits EGFR–HER2 via an intra-cellular signaling moiety which cannot be blocked by mucin or other circulating molecules which may inhibit antibody function. Given the proposed importance of EGFR–HER2 signaling in gynecologic tumors, we undertook this single agent phase II trial of lapatinib in women with advanced endometrial cancer which was recurrent or progressive despite chemotherapy. We compared clinical outcomes to translational endpoints, including receptor expression, Ki67 as a measurement of DNA synthesis, and mutations in EGFR. This was a two-stage accrual study design. After the first stage, with the inclusion of 30 evaluable patients, lapatinib demonstrated insufficient overall clinical activity in this cohort to justify going to the second stage. As EGFR inhibitors have substantial activity against endometrial cancer in preclinical models, why did the majority of patients receive no clinical benefit in this trial [19–21]?

Findings from other sites, such as breast, now shed light on the most effective use of this agent against solid tumors. Lapatinib was approved by the FDA in 2007 for use in combination with capecitabine for the treatment of HER2-positive advanced breast cancer in previously treated patients. In 2010 it was approved for use in combination with letrozole for postmenopausal women with hormone receptor positive and HER2-positive metastatic breast cancer based upon encouraging findings from clinical trials [22–24]. Importantly, previous reports

highlight that the clinical activity of lapatinib depends primarily upon the HER2 expression status of the tumor; however, the predictive status of EGFR is also currently under investigation [25].

Inclusion criteria for this trial did not require an analysis of EGFR or HER2 expression a priori; therefore, patients whose tumors were ultimately found not to express HER2 were included. Indeed, while expression of EGFR was demonstrated in the majority of tumors, only three tumors expressed HER2 on the pre-treatment biopsy. Even in these three cases, the intensity of immunostaining was low. These data are consistent with previous work indicating that only 1% of type I and 16% of type II endometrial tumors have gene amplification of HER2 and express high levels of the protein [26]. This could have been a factor underlying the lack of clinical activity of lapatinib in this study of unselected patients. However, we identified a novel EGFR mutation in exon 18, E690K, in one patient who responded. Other EGFR tyrosine kinase domain mutations have been reported to be associated with response to tyrosine kinase inhibitors, primarily in non-small cell lung cancer (NSCLC) as listed at <http://www.somaticmutations-egfr.info>. To our knowledge, this is the first time this specific mutation has been reported in the literature. Although possibly incidental, this finding, in addition to the association between higher EGFR expression and lack of progression found in this study, suggests that mutated EGFR and total EGFR may also play a role in lapatinib sensitivity.

Recent published findings also demonstrate that PTEN loss and PIK3CA mutations may predict a poor response to single agent lapatinib in breast cancer [25]. As this pathway is often constitutively activated in endometrial adenocarcinomas, which frequently harbor loss of function mutations of PTEN and gain of function mutations in PIK3CA, lack of response to lapatinib may be predictable in the setting of endometrial cancers with this common molecular phenotype [27–29].

There was a suggested association between intensity of Ki67 in recurrent, pre-treatment biopsies and response. Ki67 staining has also been positively associated with clinical response to front-line therapy in breast cancer patients, suggesting that robust cell proliferation prior to treatment may make tumors more sensitive to therapy [30]. Post-treatment biopsies were not obtained in the majority of patients on this trial, so it is not possible to know whether Ki67 levels decreased in post-treatment compared to pre-treatment tumor samples, as might be predicted for responders.

In summary, we found that lapatinib had insufficient clinical activity to warrant its use as a single agent in this cohort of unselected patients with advanced or recurrent endometrial cancer. These findings could potentially be explained by the lack of sufficient HER2 expression in advanced endometrial cancer in these cases. However, these findings may not apply to patients with HER2 amplification or certain mutations in EGFR. The tantalizing finding of a novel tyrosine kinase domain EGFR mutation, E690K, in the patient with a response suggests that molecular profiling of endometrial tumors may yet identify patients who could benefit from lapatinib treatment.

Conflict of interest statement

The co-authors have no conflicts of interest to declare.

Acknowledgments

The authors would like to thank Sandra Dascomb for the management of clinical data, the GOG Tissue Bank for their assistance with the banking and distribution of specimens, Dr. Betsy Bove and Mr. Frank Zambito for technical assistance, Leah Madden for the administration of the protocol, and Kim Blaser for publications. We also thank Anna Holmes in the GOG Core Laboratory for Receptors and Targets for technical assistance.

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