Eastern Cooperative Oncology Group Phase II Trial of Lapatinib in Men with Biochemically Relapsed, Androgen Dependent Prostate Cancer

Glenn Liu, MD[1], **Yu-Hui Chen, MS**[2], **Jill Kolesar, Pharm D**[1], **Wei Huang, MD**[1], **Robert DiPaola, MD**[3], **Michael Pins, MD**[4], **Michael Carducci, MD**[5], **Mark Stein, MD**[3], **Glenn J. Bubley, MD**[6], and **George Wilding, MD**[1]

[1]University of Wisconsin Carbone Cancer Center, Madison, WI

[2]Dana Farber Cancer Institute, Boston, MA

[3]The Cancer Institute of New Jersey, New Brunswick, NJ

[4]University of Illinois College of Medicine, Chicago, IL

[5]Johns Hopkins Kimmel Cancer Center, Baltimore, MD

[6]Beth Israel Deaconess Medical Center, Boston, MA

Abstract

Purpose—Activation of the epidermal growth factor pathway is important in prostate cancer development and the transcription of androgen receptor regulated genes. This study evaluated the potential activity of lapatinib in men with biochemically-relapsed androgen-dependent (stage D0) prostate cancer.

Experimental Design—Patients with a rising PSA after primary therapy for prostate cancer were enrolled. A PSA doubling time (PSADT) <12 months was required. Lapatinib was administered at 1,500 mg orally daily. Outcome measures were changes in PSA kinetics. Primary tumor blocks were obtained and assessed for EGFR expression, EGFR Q787Q polymorphism, and *Kras* 38 mutational status.

Results—49 patients were enrolled (14 ineligible), resulting in 35 pts for analysis. No PSA response was observed; best response was stable disease (n=28, 80.0%). Pre-treatment average slope was 0.19 log (PSA)/month (PSADT=3.70 months), in contrast to on-treatment average slope of 0.13 log (PSA)/month (PSADT=5.44 months) using linear mixed effects models (p=0.006). Median progression-free survival (PFS) was 17.4 months for the high EGFR group and 6.0 months for the low EGFR group (p=0.50). Patients with *Kras* 38 mutation had shorter PFS than those without *Kras* 38 mutation (p=0.09).

Corresponding Author: Glenn Liu, MD, University of Wisconsin Carbone Cancer Center, 1111 Highland Avenue, Wisconsin Institute for Medical Research, Room 7051, Madison, WI 53705. Office: 608-265-8689; Fax: 608-265-0614; gxl@medicine.wisc.edu.

Conclusion—Although no PSA responses (primary endpoint) was observed, lapatinib may have biologic activity in men with stage D0 prostate cancer as evidenced by a decrease in PSA slope in this non-randomized study. Additional trials assessing the role of EGFR overexpression and *Kras* wild type status in prostate cancer should be investigated.

Keywords

Epidermal growth factor receptor; tyrosine kinase inhibitors; clinical trial

INTRODUCTION

Prostate cancer is the most common diagnosed solid malignancy, and second leading cause of cancer-related mortality, among men in the United States[1]. Although most men with localized disease are cured with surgery or radiation, approximately one third of patients will develop recurrent disease manifesting initially as a rise in their prostate specific antigen (PSA)[2]. While it can take years before a patient with a rising PSA develops radiographic metastases, for patients with a PSADT of less than 12 months, this time is much shorter[3]. For those that progress or are not candidates for salvage approaches, early androgen-deprivation therapy (ADT) or watchful waiting is acceptable. Given negative effects ADT on bone density and cardiovascular health, many men avoid ADT for a rising PSA alone. Having an alternative therapy for patients with a rising PSA and no radiographic evidence for metastasis (stage D0) is desirable.

Epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) are related peptides that bind the epidermal growth factor receptor (EGFR). Over-expression of EGFR is reported in a number of epithelium-derived carcinomas and linked with aggressive tumor growth, earlier disease progression, poorer survival, and decreased response to therapy[4]. The expression of EGFR has been found in normal prostatic epithelium, prostatic intraepithelial neoplasia (PIN), and malignant prostatic cells[5]. While EGFR functions to maintain prostate epithelial integrity during embryonic development, increased EGFR signaling in the adult contributes to prostate carcinogenesis[6]. Increased EGFR signaling can occur as a result of EGFR over-expression, mutations, or interactions with other signaling pathways such as the androgen receptor (AR)-mediated pathway[7]. HER-2 has been extensively studied in breast cancer and its expression is correlated with a more aggressive phenotype and less responsiveness to hormonal agents[8]. While agents targeting HER-2, such as trastuzumab[9], have proven clinical benefit in breast cancer, its role in prostate cancer remains unclear. Xenograph studies have shown activity of trastuzumab against the androgen-dependent prostate cancer cell line CWR22 and LNCaP, but not against androgen independent cell lines[10]. Other agents inhibiting EGFR have also shown encouraging preclinical activity against prostate cancer including gefitinib[11]. There is strong *in vitro* evidence that interaction of growth factors and androgens is important in prostate cancer development. EGF and other peptide growth factors induce the transcription of AR-regulated genes. Additionally, AR activation in the presence of low levels of androgens is dramatically increased by the presence of these peptide growth factors[12]. Therefore, targeting EGFR function is reasonable approach in prostate cancer.

Lapatinib (GW572016, NSC 727989, Tykerb®) is a selective dual inhibitor of EGFR and ErbB2 tyrosine kinase activity[13]. Lapatinib is thought to react with the ATP binding site of EGFR/ErbB2, resulting in inhibition of autophosphorylation and subsequent proliferative signaling[14]. Studies suggest that inhibition of EGFR by lapatinib results preferentially in cell growth arrest, while inhibition of ErbB2 leads to cell growth arrest and apoptosis[15].

Unfortunately, human trials in castrate-resistant prostate cancer with trastuzumab and gefitinib have not shown substantial clinical activity[16,17]. Newer EGFR inhibitors that target multiple EGFRs simultaneously may be more effective than those targeting EGFR or ErbB2 alone. Likewise, preclinical data suggest that targeting EGFR may be more effective against androgen-*dependent* disease[10]. Here we report the results of a Phase II trial of the dual EGFR and ErbB2 tyrosine kinase inhibitor lapatinib in androgen-dependent prostate cancer.

PATIENTS AND METHODS

Patients

Patients with histologically proven prostate cancer previously treated with surgery and/or radiation therapy, now with progressive disease evidenced by a rising PSA (defined as a reference PSA value followed by two rising PSA values, each higher than the previous value, obtained at least 6 weeks apart, all at the same reference laboratory and within 6 months of registration) were enrolled. Minimum PSA value at registration must be greater than 0.4 ng/mL if prior prostatectomy or 1.5 ng/mL if prior radiotherapy only. No evidence of metastatic disease on bone scintigraphy, computed tomograpgy (CT), magnetic resonance imaging (MRI) or physical examination was permitted. Prior neoadjuvant/adjuvant androgen-deprivation therapy was allowed if discontinued greater than 1 year prior to enrollment with serum testosterone level >150 ng/dL within 4 weeks of registration. Prior vaccine/immunotherapy for prostate cancer was not allowed. Other agents such as 5-α reductatse inhibitors, ketoconazole, megestrol acetate, systemic steroids, or herbal supplements were not allowed during the period that PSA values were being obtained for eligibility. A PSADT of <1 year (365 days) was required using the following formula:

$$PSADT \text{ in days} = 0.693 \text{ (t) } / \text{ } [\ln(PSA_2) - \ln(PSA_1)]$$

t = number of days between PSA_1 and PSA_2

ln = natural log

PSA_1 = middle PSA evaluation

PSA_2 = last PSA obtained for eligibility.

Adequate hematologic (leukocytes 3000/mm^3, granulocytes 1500/mm^3, platelets 100,000/mm^3), renal (normal serum creatinine or creatinine clearance 60 mL/min/1.73 m^2), liver (normal serum total bilirubin and alkaline phosphatase, SGOT (AST) and SGPT (ALT) 2.5 × institutional upper limit of normal) function was required. Other eligibility criteria included: normal cardiac ejection fraction by echocardiogram (ECHO) or multigated acquisition scan (MUGA) within 4 weeks of registration; no concomitant use of any potent CYP3A4 inducer or inhibitor; no active gastrointestinal tract disease; Eastern Cooperative Oncology Group Performance Status 0 or 1; age 18 years. All patients provided written informed consent. This study was approved by the ethics committee or institutional review board at each center and complied with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations.

Study Design and Treatment Plan

This multi-institutional phase II was conducted through the Eastern Cooperative Oncology Group. The primary endpoint was 50% decline in PSA using Prostate Specific Antigen Working Group (PSAWG 1) criteria[18]. All patients received lapatinib at a starting dose of 1,500 mg daily, administered orally on an empty stomach, in 28 day cycles. Patients continued on therapy until PSA progression (defined as increase in PSA by 50% over nadir or baseline, confirmed by a repeat PSA two weeks later, with an absolute increase in PSA of at least 5 ng/mL), clinical progression (appearance of new lesions on CT, MRI, or bone

scintigraphy), or unacceptable toxicity. Up to two dose reductions (1,000 mg/day and 750 mg/day) were allowed (required for any grade 3 or greater toxicity attributed to lapatinib using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0 or subjectively intolerable recurrent grade 2 events). Patients were assessed at the beginning of each new cycle of therapy (every 28 days) with a history, physical examination, basic chemistry and hematologic laboratories, and PSA. Repeat ECHO/MUGA scans were conducted every two cycles with repeat bone scintigraphy and CT scans of the abdomen and pelvis repeated every 6 months (sooner if clinically indicated).

Response evaluation

PSA values were collected monthly. A PSA response was defined by a   50% reduction in PSA, compared to baseline, confirmed by a repeat PSA at least 4 weeks later. PSA progression was defined by an increase in PSA value by at least 50% over nadir or baseline value, confirmed by a second PSA two weeks later, with an absolute increase at least 5 ng/mL. Stable disease was defined as neither meeting response nor progressive disease criteria for at least 3 cycles of therapy. Progression-free survival (PFS) was defined as the time from registration to first progression of any type (clinical, radiographic, or PSA) or death, whichever came first.

Correlative analysis

Peripheral blood from each patient was drawn into Paxgene tube and DNA was extracted using PreAnalytix Blood DNA system (Qiagen, Valencia, CA) according to the manufacturer's protocol. Diagnostic blocks from prostatectomy were used to obtain tumor DNA by laser capture microdisssection on paraffin-embedded formalin-fixed sections as previously described[19,20]. DNA from peripheral blood mononuclear cells (PBMCs) was used for genotyping single nucleotide polymorphisms and tumor tissue was used for mutation analysis.

Pyrosequencing and allele quantitation—Pyrosequencing was used to assess *Kras* mutation in tumor tissue and drug metabolizing SNPs in DNA obtained from PBMCs as previously described[21,22].

Automated quantitative analysis—Paraffin embedded prostate cancer tissue was collected at baseline to analyze EGFR expression using automated quantitative analysis (AQUA)[23,24]. Rabbit E-cadherin (1:250, Abcam) and rabbit anti-CK WSS (1:250, Dako) cocktail and Alexa Flour 555 conjugated goat anti-rabbit IgG (1:200, Invitrogen) were used to define and visualize epithelial compartment. EGFR mouse monoclonal antibody (1:200, Biocare Medical), biotinylated goat anti-mouse (Biocare Medical), streptavidin-HRP and Alexa Fluor 647 tyramide (1:50, Invitrogen) were used to detect and visualize EGFR. 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen) was used to define and visualize nuclear compartment.

Statistical Analysis

The primary endpoint of interest was proportion of patients with PSA response defined as a 50% or greater decline in serum PSA level. An underlying true response rate of 20% was considered clinically significant whereas a true response rate of 5% would be of no clinical interest. The accrual goal of this study was 50 patients (45 eligible). If 6 or more responses were observed among the 45 eligible patients, the protocol treatment would be considered worthy of further study for this patient population. With this design, the probability of concluding that the treatment is effective was 0.91 if the true response rate was 20% and 0.02 if the true response rate was 5%.

Descriptive statistics were used to characterize patients at study entry. Exact binomial confidence intervals[25] were used to describe responses. The method of Kaplan and Meier[26] was used to characterize overall survival and PFS. A linear mixed-effects model[27] was used to study changes in PSA over time. Fisher's exact test[25] and the Cochran-Armitage[28] trend test were used to assess the associations between polymorphisms and lapatinib associated toxicities. The log rank test[29] was used to evaluate the associations between PFS and polymorphisms, mutations, as well as EGFR expression. No adjustment for multiple comparisons was performed. All p-values are two-sided, and p-values less than 0.05 are considered statistically significant.

RESULTS

Patient characteristics

Between September 29, 2005 and July 5, 2006, 49 patients were registered from 14 separate ECOG institutions. While all treated patients were evaluable for safety and toxicity assessments, 14 patients were eventually determined to be ineligible for the efficacy assessment resulting in 35 evaluable patients for the primary analysis. The reasons for ineligibility included use of baseline PSA values > 6 months from registration (n=5), use of eligibility PSA values < 6 weeks apart (n=2), PSADT > 365 days (n=2), baseline PSA values obtained after registration (n=2), elevated serum creatinine level (n=2), and use of prior vaccine (n=1). Patient characteristics are shown in Table 1.

PSA response and kinetics

No PSA responses were observed. Stable disease was experienced by 28 (80.0%; 90% CI: [65.7%, 90.2%]). Progressive disease was the best response in 4 (11.4%) patients. Three patients were unevaluable for PSA response (1 patient received 4 days of therapy and was removed from study due to an adverse event; 2 patients received < 3 cycles of therapy and thus were not evaluable for response per protocol specified criteria).

PSA slopes were assessed using multiple PSA values prior to registration and during treatment. The one patient that received only 4 days of drug and did not have any on treatment PSA values for assessment and therefore was not included in this analysis. Three PSA values were obtained prior to registration (each at least 6 weeks apart, but within 6 months of registration). Post-registration PSA values were collected from all 34 eligible patients at least 2 times, at most 30 times, with a median of 6 times. Figure 1 shows the natural log-transformed PSA values for each eligible patient over time along with PSA slopes prior to registration and during treatment. The pre-registration average slope was 0.19 log (PSA)/month (PSADT=3.70 months), in contrast to on-treatment average slope of 0.13 log (PSA)/month (PSADT=5.44 months) using linear mixed effects models (p=0.006).

Overall and progression-free survival

Survival time was determined as time from study entry to death (or last known date alive). At the time of the last analysis, 5 patients had died, one refused further follow-up, and 29 of the remaining eligible patients were alive. Median follow-up was 33.4 months, with median survival not reached. The median PFS is 7.4 months with the PFS at 2 years being 16.0% (90% CI:[6.5%,29.1%]). Table 2 shows the number of cycles administered and reasons for study discontinuation for all eligible patients. The median duration of treatment was 5.5 months. One patient remains on study with over 32 cycles of therapy.

Safety and tolerability

All patients who received protocol therapy, regardless of eligibility, were evaluable for toxicity. No CTCAE grade 4 or higher adverse events were observed. All toxicities were

mild or moderate in severity, except for 4 cases of grade 3 events (fatigue, AST elevation, 2 cases of hyperbilirubinemia). The most common toxicities were acneiform rash (71.4%), diarrhea (67.3%), and fatigue (44.9%). Of note, one decrease in left ventricular ejection fraction (grade 2) was observed.

Exploratory analyses

Tissue blocks were available and suitable for EGFR expression analysis and *Kras* 38 mutational status in 14/35 eligible patients. Of these, 4 had high and 10 had low EGFR expression levels. The median PFS (Figure 2) for high EGFR group was 17.4 months versus 6.0 months in the low EGFR group (p = 0.50). Patients with *Kras* 38 mutation (Figure 3) had a median PFS of 4.7 month versus 12.4 month for wild type *Kras* 38 (p=0.09). Figure 4 shows waterfall plot of the change in PSADT for each patient, with EGFR expression level and *Kras* status indicated where available. Peripheral blood mononuclear cells were available in 28/35 patients. The EGFR Q787Q AA allele was identified in 9, AG allele in 14, and GG allele in 5 patients. No correlation with PFS was evident due to small sample size.

The presence of drug metabolizing enzyme polymorphisms (MDR1 1236, MDR1 2677, MDR1 C3435T, CYP3A4, CYP 3A5, CYP 3A43, and CYP2C8) were assessed and not found to be associated with lapatinib toxicity (e.g. rash or diarrhea) using Fisher's exact test. No significant association was identified in an additive genetic model using the Cochran-Armitage trend test.

DISCUSSION

Rising serum PSA after primary therapy implies persistent disease and represents a growing population of patients seeking treatment at large academic centers in the United States. Although androgen-deprivation therapy is acceptable therapy, observation is also reasonable to minimize treatment-related morbidity. As a result, the clinical investigation of newer agents in this setting is of interest, although challenging as the only indicator of active disease is a rising PSA. There is debate regarding the utility of PSA changes, especially with newer targeted agents, and the PSA Working Group has advocated using radiographic progression-free survival as a preferred endpoint for Phase 2 trials[30]. While this is reasonable in the metastatic, castrate-resistant setting, this endpoint in stage D0 prostate cancer may not be practical. Others have argued that changes in PSADT may be a markers of drug effect, understanding that shorter PSADT correspond to worse prognosis and thus a favorable change in PSADT suggest drug activity[31,32]. On the other hand, PSADT changes may simply be affected by differences in timing of PSA collections during the pre-therapy period compared to the to on-treatment period[33]. Thus, these PSADT changes in the D0 population must be interpreted cautiously.

We report the phase II results of single-agent lapatinib in men with stage D0 prostate cancer. Lapatinib was well tolerated with mild to moderate diarrhea and rash as expected for this class of drug. While the primary endpoint for statistical purposes was PSA response rate (as this was the standard method of reporting outcomes at the time this trial was designed and mandated by the Cancer Therapy Evaluation Program), secondary efficacy endpoints included changes in PSA kinetics (e.g. PSADT). Understanding the challenges mentioned above, we attempted to carefully and stringently collect multiple pre-therapy PSA values in order to accurately calculate PSADT changes pre- and during lapatinib exposure. The difficulty in assuring strict PSA acquisitions for eligibility in a multi-institutional study is evident by the high number of patients (n = 11) that did not have PSA values collected as specified, and thus were deemed unevaluable for the planned PSADT analysis. Much of this could be explained by unfamiliarity with having PSADT eligibility criteria. While no PSA

responses were observed, we did see a statistically significant increase in PSADT (from 3.70 months pre-therapy to 5.44 months on-treatment) (p = 0.006).

The importance of EGFR expression[34,35], EGFR mutational status[36], and the *Kras* 38 mutational status[37] has been identified as important indicators in predicting drug activity to EGFR inhibitors in other malignancies. While change in PSADT by itself is not definitive evidence of clinical activity in this non-comparative study, when considering the observed differences in median PFS, stratified by low/high EGFR expression (Figure 2) and presence/absence of a *Kras* 38 mutation (Figure 3), the potential activity of lapatinib is suggested. Needless to say, the small sample size makes any conclusion speculative, but nonetheless, hypothesis generating. No significant association between the presence of *Kras* 38 mutation status with EGFR expression was observed in our study. Of note, no correlation between change in PSADT and with common EGFR-mediated toxicities such as rash or diarrhea was evident.

The androgen receptor remains the primary target in this disease. Unfortunately, all patients eventually develop castrate-resistant prostate cancer with current therapies. Strategies to modulate AR-signaling are being investigated. One approach targets the cross talk between EGFR signaling pathways with AR-signaling pathways. It has been hypothesized that inhibiting EGFR may be important in blocking ligand-independent pathways that activate AR signaling. Unfortunately, trials to date have not resulted in any clinically significant activity in men with *castrate-resistant* disease[16,17].

Preclinical data supports that in the presence of physiologic androgen exposure, EGFR expression on prostate cancer cells will increase, allowing both EGF and TGF-α to stimulate further prostate cancer cell proliferation[38]. This suggests that prostate cancer cell growth is regulated not only by AR activation, but that prostate cancer progression, in the androgen-dependent state, utilizes additional peptide growth factor pathways, such as EGF. If true, the targeting EGFR in androgen-dependent prostate cancer may be of higher clinical utility. To the best of our knowledge, our trial is the only study assessing EGFR inhibitors in men with androgen-*dependent* prostate cancer.

Here we report the results of a small single-arm phase 2 trial assessing activity of lapatinib in men with biochemically relapsed, androgen-dependent prostate cancer. Although this study did not meet its mandated primary endpoint (PSA response), lapatinib appears to have some potential antitumor activity in androgen-dependent prostate cancer evident by increases in PSADT. The suggestions that EGFR expression and *Kras* status may impact benefit with lapatinib in this patient population deserves further investigation. The only way to address this issue clinically is with a randomized trial.

Acknowledgments

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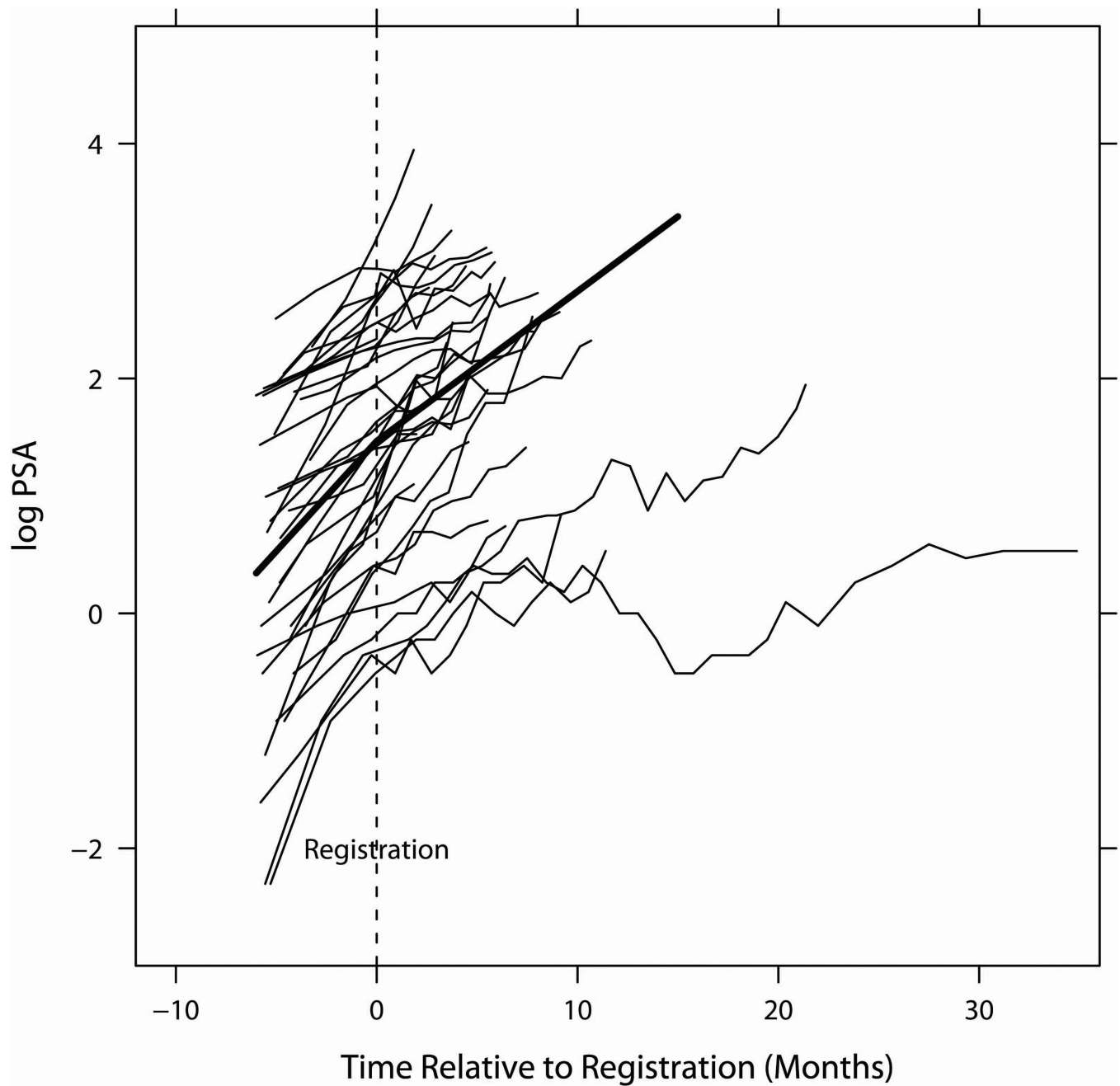
Figure 1.
Estimated population average PSA slopes (thicker line) and individual patient changes in PSA over time (thin lines) among all eligible patients.
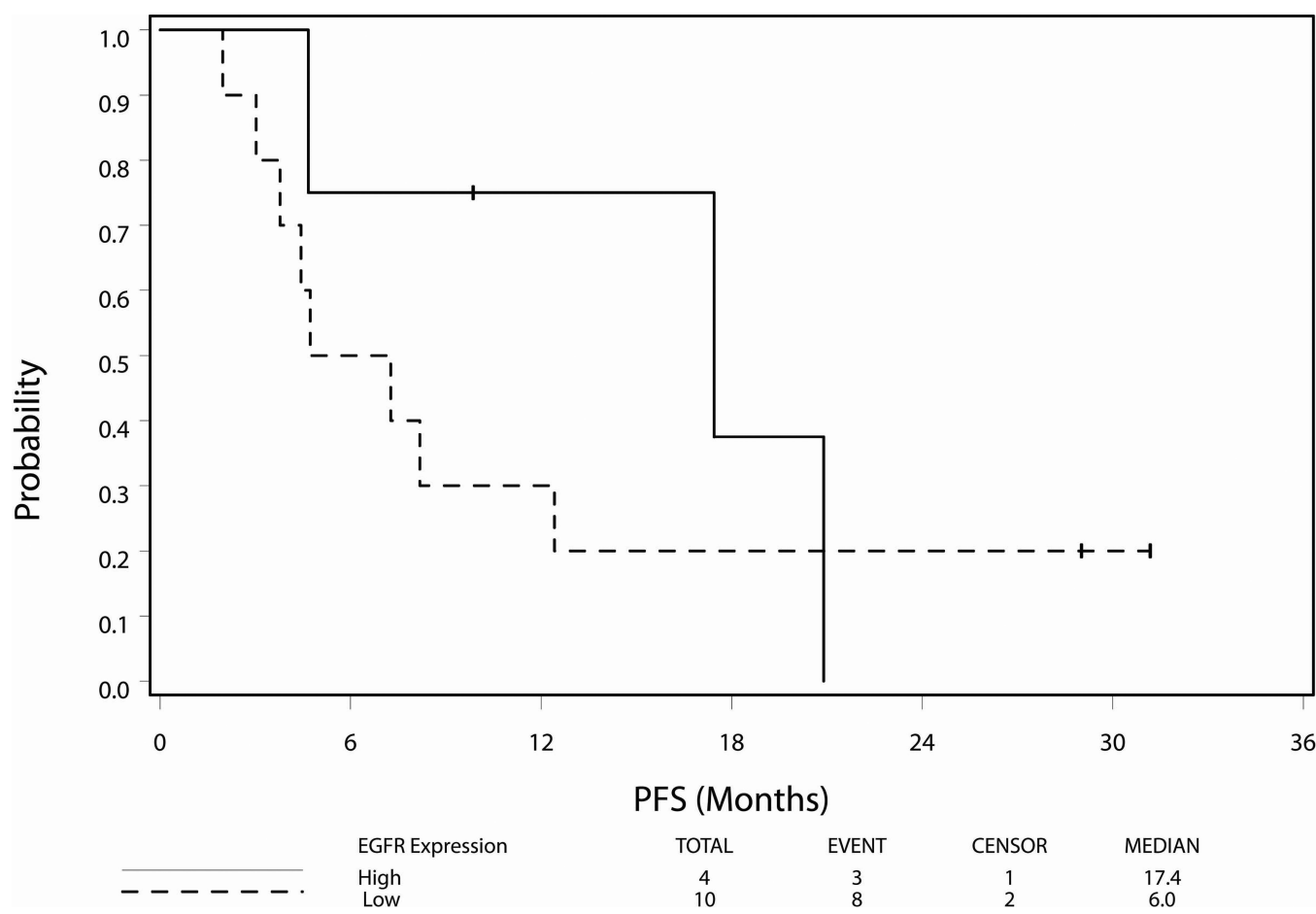
EGFR Expression	TOTAL	EVENT	CENSOR	MEDIAN
High	4	3	1	17.4
Low	10	8	2	6.0

Figure 2.
Progression-free survival by EGFR expression level

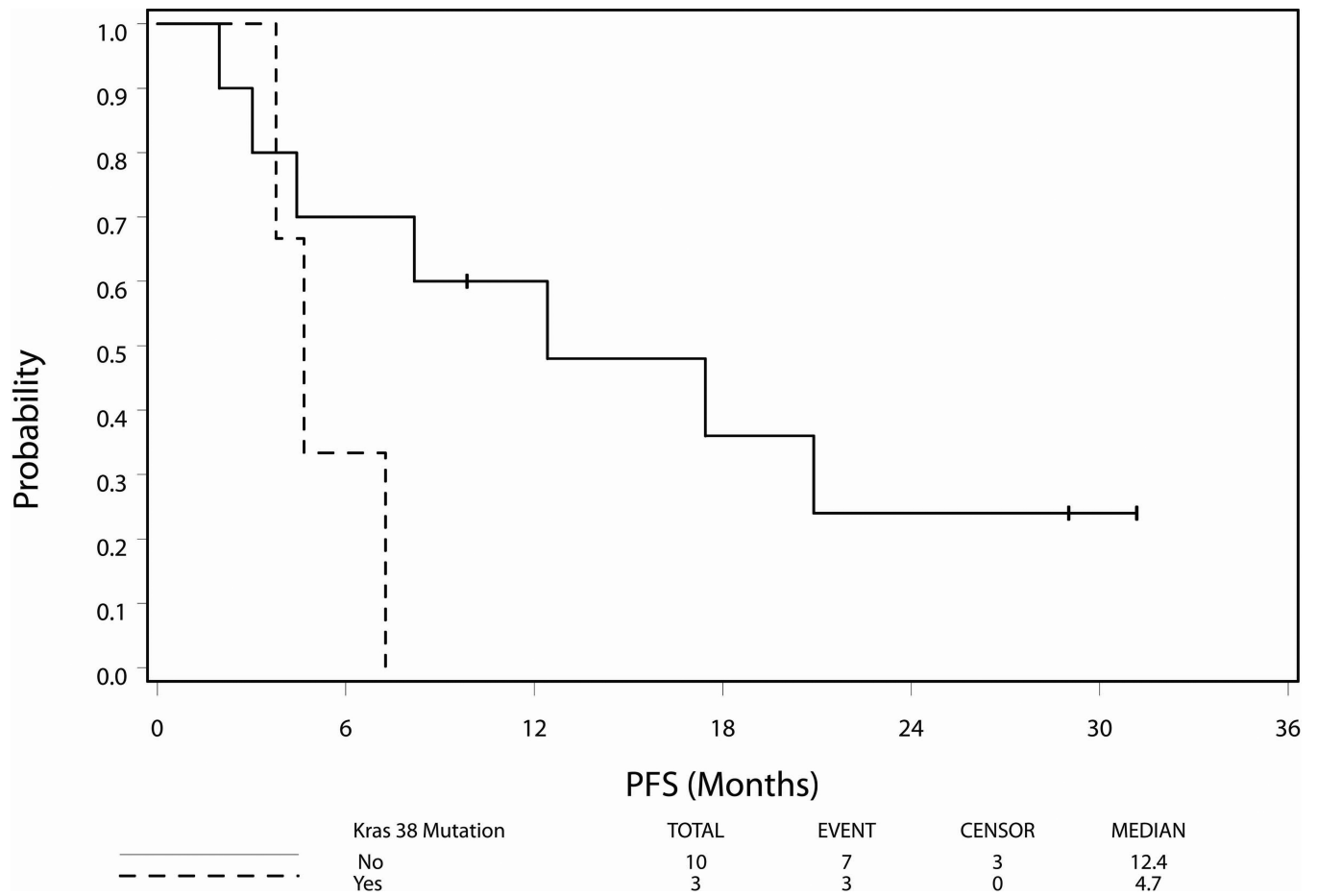| Kras 38 Mutation	TOTAL	EVENT	CENSOR	MEDIAN
No	10	7	3	12.4
Yes	3	3	0	4.7

Figure 3.
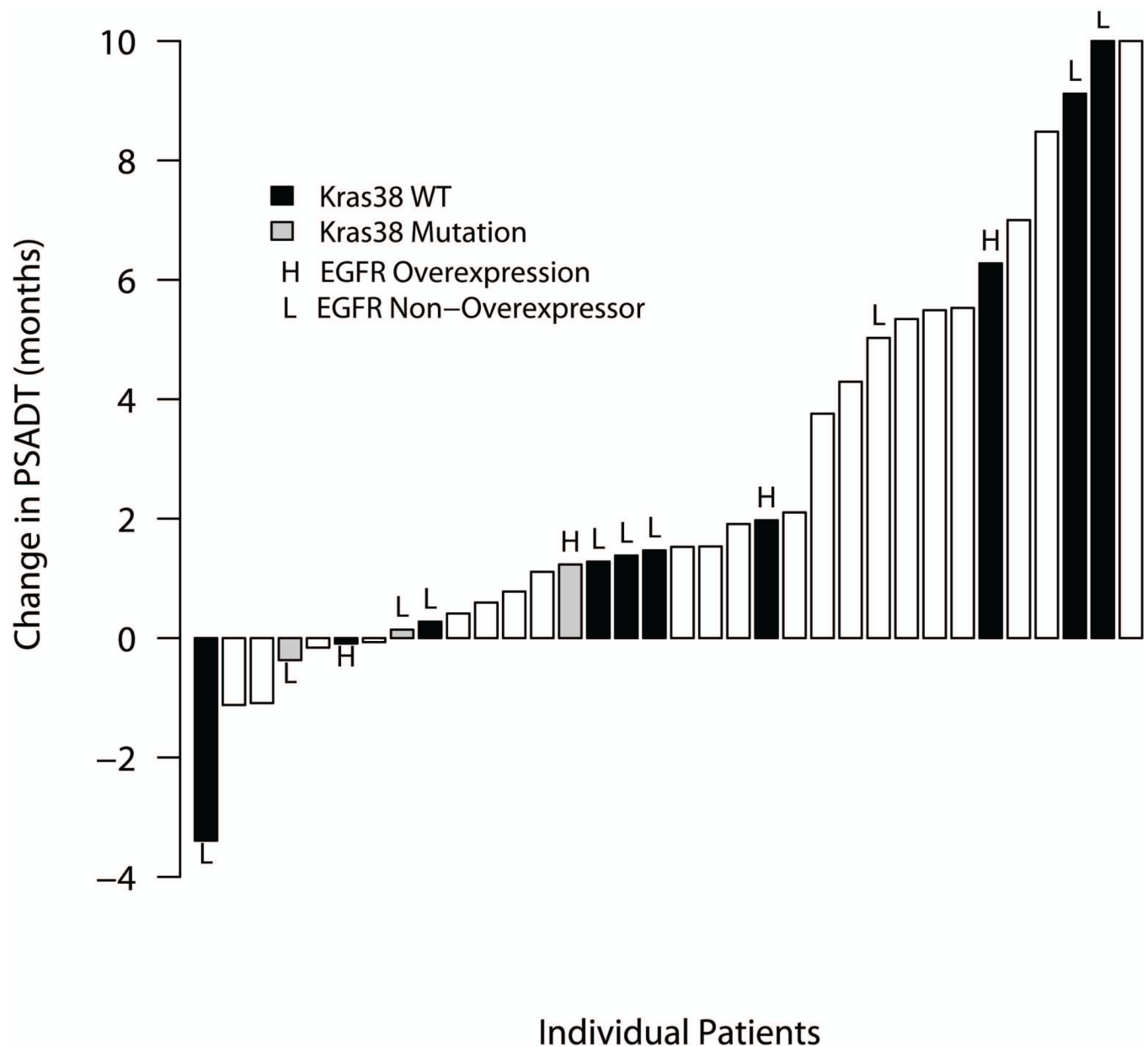Progression-free survival by Kras 38 mutational status

Figure 4.
Change in PSADT (months) from pre-registration to on-study. Of note, the change in PSADT for the two patients shown on the far right were much greater than 10 months (one had a negative PSADT and the other a PSA slope near zero). Where available, the EGFR expression level (high or low) and *Kras* status (wild type or mutant) are included. *Bars above the line reflect increases in PSADT, whereas bars below reflect a decrease.*

Table 1

Patient demographics and characteristics

Characteristic	No.	Percent
Median age: years (range)	65.0 (48–81)	
Race		
Caucasian	33	94.3%
African American	2	5.7%
Pathologic T stage at diagnosis		
T2a	3	8.6%
T2b	10	28.6%
T3a	7	20.0%
T3b	9	25.7%
Tx	6	17.1%
Pathologic N stage at diagnosis		
N0	26	74.3%
N1	3	8.6%
Nx	6	17.1%
Gleason Score		
6	7	20.0%
7	15	42.9%
8–10	12	34.3%
Unknown	1	2.9%
Prior therapies		
Prostatectomy	29	82.9%
Radiation therapy [*]	27	77.1%
Androgen-deprivation therapy	8	22.9%
Other therapies	3	8.6%

[*] Includes primary (6 pts) and salvage (21 pts) radiotherapy.

Table 2

Number of treatment cycles received and reason for study discontinuation.

Reason for study discontinuation	Cycles				Total
	1–5	6–10	11–15	>15	
Progressive disease	5	9	3	1	18
Adverse events	2	1	0	0	3
Patient withdrawal	6	3	0	0	9
Alternative therapy	0	1	0	0	1
Complicating disease	0	1	0	0	1
Other	0	0	2	0	2
Total	13	15	5	1	34 *

*
One patient remains on treatment, and thus not included in this table as he has not yet met criteria for study termination.