Lung Cancer 64 (2009) 51–59

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Pilot study of gefitinib and fulvestrant in the treatment of post-menopausal women with advanced non-small cell lung cancer

Anne M. Traynor[a,*], Joan H. Schiller[b], Laura P. Stabile[c], Jill M. Kolesar[a], Jens C. Eickhoff[a], Sanja Dacic[d], Tien Hoang[a], Sarita Dubey[e], Sarah M. Marcotte[a], Jill M. Siegfried[c]

[a] University of Wisconsin Paul P. Carbone Comprehensive Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States
[b] Division of Hematology and Oncology, Department of Internal Medicine, University of Texas at Southwestern Medical Center, Dallas, TX, United States
[c] Department of Pharmacology, Lung and Thoracic Malignancy Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA, United States
[d] Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States
[e] University of California at San Francisco Cancer Center, University of California at San Francisco School of Medicine, San Francisco, CA, United States

ARTICLE INFO

Article history:
Received 11 March 2008
Received in revised form 30 June 2008
Accepted 2 July 2008

Keywords:
Epidermal growth factor receptor
Hormonal treatment
Non-small cell lung cancer
Sex
Age
Estrogen receptor

ABSTRACT

Introduction: Estrogen receptor beta (ERβ) has been detected in non-small cell lung cancer (NSCLC) cell lines and tumor specimens. The ER down-regulator, fulvestrant, blocked estradiol-stimulation of tumor growth and gene transcription in NSCLC preclinical models and showed additive effects with the epidermal growth factor receptor (EGFR) inhibitor gefitinib. The safety and tolerability of combination therapy with the EGFR inhibitor, gefitinib, and fulvestrant was explored.
Methods: Post-menopausal women with advanced NSCLC received gefitinib 250 mg po daily and fulvestrant 250 mg IM monthly.
Results: Twenty-two patients were enrolled. Eight patients had adenocarcinoma, six NSCLC-NOS, four squamous cell, and four BAC. Seven patients were never-smokers. Eight patients received ≥2 lines of prior chemotherapy, six received one prior chemotherapy, and eight were treatment-naïve. One patient experienced grade 4 dyspnea possibly related to treatment; all other grade 3/4 toxicities were unrelated to treatment. Twenty patients were evaluable for response: three partial responses (PRs) were confirmed (response rate of 15%, 95% CI: 5–36%). The median progression-free survival (PFS), overall survival (OS), and estimated 1-year OS were 12 weeks (3–112 weeks), 38.5 weeks (7–135 weeks), and 41% (95% CI: 20–62%), respectively. Survival outcomes did not differ by prior lines of therapy. A subset analysis revealed that OS in the eight patients whose tumors exhibited at least 60% ERβ nuclear IHC staining measured 65.5 weeks, while that of the five patients with ERβ staining of less than 60% was 21 weeks. One patient with bronchioalveolar carcinoma (BAC) and a PR had an EGFR L858R mutation in exon 21. There was no correlation between ERβ IHC expression and histology or smoking history.
Conclusions: Combination therapy with gefitinib and fulvestrant in this population was well tolerated and demonstrated disease activity.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality in women in the US, comprising 25% of all cancer-related deaths [1]. Certain clinical characteristics distinguish lung cancer in women: a higher percentage of women, compared to men, are diagnosed under the age of 50, non-smokers diagnosed with lung cancer are more likely to be women, women are more likely than men to be diagnosed with adenocarcinoma or bronchioalveolar histologies, and survival is superior for women for all stages of disease, even after controlling for treatment [2–5].

Epidemiologic studies in smokers have yielded conflicting results as to whether sex impacts the risk of developing lung cancer [6–9]. Multiple mechanisms may account for any excess risk of developing lung cancer in women. For example, female smokers have been shown to exhibit expression of gastrin-releasing peptide receptor (GRPR) mRNA at a lower mean pack-year tobacco exposure than male smokers. Gastrin-releasing peptide receptor is the receptor for gastrin-releasing peptide, a bombesin-like peptide that stimulates cell proliferation and acts as an autocrine growth factor in lung cancer [10]. The gene for GRPR is located on the X chromosome near a cluster of genes that escape X-inactivation.

* Corresponding author. Tel.: +1 608 262 5092; fax: +1 608 265 8133.
E-mail address: amt@medicine.wisc.edu (A.M. Traynor).

0169-5002/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2008.07.002

Further, patterns of polymorphisms of phase I enzymes may influence the risk of disease in women. In that regard, *CYP1A1*, an enzyme active in the conversion of carcinogens to DNA-reactive metabolites, is also involved in the metabolism of estradiol. Retrospective analyses have detected a higher frequency of DNA adducts per smoking dose and higher levels of *CYP1A1* gene expression in females, compared to males, in both lung tumor specimens and in normal lung tissue adjacent to tumor tissue [11]. Patterns of polymorphisms of *CYP1A1* have been associated with an increased risk of developing lung cancer in women [12]. Studies in normal human bronchoepithelial cells demonstrate that the estrogen receptor (ER) α enhances the expression of *CYP1B1* and *CYP1A1*, key bioactivating enzymes in the carcinogen metabolism pathway, and this may serve as a mechanism for enhanced female sex-related susceptibility to tobacco-mediated DNA mutations [13].

Lastly, hormonal mechanisms may potentially contribute to distinctions in disease risk and in the clinical characteristics of lung cancer between women and men. Hormone replacement therapy was found to be associated with shorter overall survival in women diagnosed with lung cancer, suggesting estrogen signaling might lead to lung cancer progression [14]. In contrast, hormone replacement therapy before lung cancer diagnosis appears to protect against development of an ER-expressing lung tumor [15]. These conflicting results suggest there is a shift in the balance between tumor-protective effects of estrogen, such as inducing differentiation and stimulating the immune system, and the tumor-promoting effects of estrogen, such as stimulation of growth-promoting gene expression in normal tissues compared to neoplastic tissues.

Two wild-type ER forms, ERα and ERβ, have been identified that share considerable amino acid homology in the DNA and ligand binding regions, but differ considerably in their tissue distribution [16,17]. ERα is found in the alveoli, where it plays a role in estrogen-induced alveolar regeneration and is expressed by an immortalized human bronchiolar epithelial cell line [18,19]. ERβ is highly expressed in airway epithelial tissue, in areas of atypical adenomatous hyperplasia, and in most types of lung cancer in both men and women, while lung tumors express little ERα [20]. ERβ was found to modulate the expression of platelet derived growth factor A and granulocyte-macrophage colony stimulating factor, key regulators of alveolar formation and surfactant homeostasis, respectively, in mouse models of alveolar development and function [21]. Levels of phase I and II carcinogen-activating enzymes in lung tissue were also shown to correlate with ERβ expression [22]. Estrogen receptor β has induced transcriptional responses from both an estrogen response element and an activator protein (AP-1) element in lung cancer cells; administration of the ER down-regulator, fulvestrant, blocked these effects both *in vitro* and *in vivo* [23]. These results have been confirmed using a colony stimulating assay [20]. Fulvestrant, also known as ICI 182,870, is a pure ER antagonist that blocks activity of both ERα and ERβ [24,25]. Estrogen also stimulated expression of E-cadherin, cyclin D1 and ID5, genes associated with tumor progression and growth [26]. Retrospective reviews of resected non-small cell lung cancer (NSCLC) specimens from both male and female patients have demonstrated ERβ staining per IHC in at least 45% of tumors. Multivariate analyses have shown that the presence of ERβ is a positive prognostic variable, although in two of these four series, this favorable association was restricted to male patients [27–30].

Inhibition of the epidermal growth factor receptor (EGFR) and its downstream effectors represents an important therapeutic target in the treatment of NSCLC and other epithelial malignancies. Monotherapy with erlotinib, a small molecule inhibitor of the EGFR tyrosine kinase activity, yielded prolonged survival and improved symptom control in patients with advanced NSCLC, compared to supportive care, resulting in FDA approval for this indication [31]. Prospective and retrospective studies have attempted to identify predictors of clinical benefit from EGFR inhibitors; examples of these have included absence of smoking history, Asian ethnicity, adenocarcinoma histology, EGFR protein expression, the presence of somatic EGFR mutations, amplification of gene copy number, and the severity of treatment-related rash [31–36]. Although early reports with these agents identified female sex as being associated with radiographic response to treatment, sex did not fall out as a predictor of survival in the only placebo-controlled monotherapy experience with an EGFR blocking agent [31,37]. Investigation of this class of agents remains robust in the treatment of patients with NSCLC, including means to optimize their combination with cytotoxics, other targeted agents, and as radiosensitizers [38–40].

The objective of our therapeutic combination was to exploit the potential interactions between the estrogen and EGFR pathways in patients with progressive lung cancer. Bidirectional signaling between the ER and EGFR pathways has been documented in breast and ovarian cells, and has recently come under investigation in lung cancer models [23,41–44]. In addition to nuclear ER activation, a nonnuclear ER pool has been proposed that can exert its effect via rapid signaling through various kinase cascades, including the EGFR pathway and its downstream effectors, such as MAPK [23,41]. Stabile et al. demonstrated that EGFR protein expression was up-regulated in response to anti-estrogens *in vitro*, and that ERβ expression was decreased in response to EGF and was increased in response to the EGFR tyrosine kinase inhibitor (TKI), gefitinib [23]. This work suggests that the EGFR pathway is more activated when estrogen is depleted in lung cancer cells, establishing a rationale for this combined therapy. Additionally, Stabile and others have shown *in vitro* and *in vivo* that the combination of fulvestrant and an EGF TKI in lung cancer models can maximally inhibit cell proliferation, induce apoptosis, and affect downstream signaling pathways [23,41]. Therefore, we conducted a pilot study of gefitinib, an EGFR TKI, in combination with the anti-estrogen, fulvestrant, in post-menopausal women with progressive NSCLC, in order to assess the safety and tolerability of this novel combination and to explore molecular predictors of response and toxicity.

2. Patients and methods

2.1. Patient selection

Patients with pathologically confirmed advanced (stage IIIB with pleural or pericardial effusion, stage IV, or recurrent) NSCLC who gave informed consent according to institutional and Food and Drug Administration guidelines were eligible for this study provided that the following criteria were met: ECOG performance status (PS) of 0, 1, or 2; brain metastases, if present, must have been clinically stable after treatment with surgery and/or radiotherapy; subjects must have been female and post-menopausal, defined as a woman fulfilling any one of the following criteria: age ≥60 years, age ≥45 years with amenorrhea ≥12 months with an intact uterus, having undergone a bilateral oophorectomy, or FSH levels in post-menopausal range (utilizing ranges from the testing laboratory facility); adequate bone marrow, liver and renal function; PT/INR and PTT within the upper limit of institutional normal (low dose warfarin prophylaxis was permissible); no prior therapy with gefitinib, fulvestrant, or an aromatase inhibitor; life expectancy of at least 3 months; no use of estrogen replacement therapy within 4 weeks of registration and must have agreed to remain off for the duration of the study; and no clinical diagnosis of active interstitial lung disease. Patients who previously received chemotherapy or radiotherapy treatment for their NSCLC were eligible to participate.