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Targeting the estrogen pathway for the treatment and prevention of lung cancer

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SUMMARY

The estrogen signaling pathway is involved in the biology of non-small-cell lung cancer and represents a novel therapeutic target for lung cancer. This is supported by epidemiological evidence, preclinical studies and recent data from clinical trials. Antiestrogens and inhibitors of estrogen synthesis have been shown to inhibit lung tumor growth as well as prevent lung tumorigenesis in preclinical models both *in vitro* and *in vivo*. Two clinical trials testing the effectiveness of hormonal strategies in advanced non-small-cell lung cancer have recently been completed with promising results. Future work in this field should focus on identification of patients that would benefit from hormone modulators so that they can be used earlier in the course of disease or for chemoprevention.

Lung cancer is the leading cause of cancer death in the USA and worldwide. In 2013 alone, an estimated 228,190 US cases will be diagnosed and 159,480 US deaths are estimated to occur [1]. The 5-year survival for all lung cancer patients is a dismal 15% [2]. Clearly there is need for novel therapeutic approaches in lung cancer. Over the last decade, increasing evidence from multiple disciplines has demonstrated that the estrogen signaling pathway plays an important role in lung tumorigenesis (Figure 1). Furthermore, recent data has suggested that antiestrogen therapy may be an effective therapeutic strategy for advanced stage lung cancer [3,4]. In this report, we will review the available epidemiologic, patient tumor data, preclinical and finally clinical data on antiestrogen therapy for non-small-cell lung cancer (NSCLC).

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Estrogen & lung cancer

Sex differences in lung cancer risk & presentation

Sex differences in tobacco-associated lung cancer risk have been hypothesized. However, a growing body of recent literature does not support the idea that women are more susceptible to tobacco-induced lung cancer compared with men [5–15]. Smoking is the main cause of lung cancer, however other factors such as steroid hormones are thought to play a role in lung carcinogenesis. Women have several unique clinical characteristics in lung cancer presentation compared with men that may have biological relevance. For example, women are more likely than men to be diagnosed with adenocarcinoma compared with other histological subtypes, tend to be younger and to be never-smokers. Several cohort studies have demonstrated that lung cancer incidence rates in never-smokers is greater in women compared with men [16,17]. Women also have improved survival rates for all stages of lung cancer [18,19]. In addition, among patients with lung adenocarcinoma, women are more likely than men to have tumors with *EGFR* mutations, which are more responsive to EGF receptor (EGFR) tyrosine kinase inhibitors (TKIs) and may partially account for the survival advantage observed in women [20]. This observation may be related to the crosstalk between the estrogen receptor (ER) and the EGFR pathways in lung cancer [21,22]. One study has shown that women over the age of 60 years with advanced NSCLC had a survival advantage over men and younger women [23]. These observations in sex differences in lung cancer presentation and survival suggest a role for hormones in this disease. Both exogenous and endogenous estrogens may play a role.

Hormone replacement therapy & lung cancer risk

Additional evidence for a role of estrogens in this disease comes from several population studies that examined hormone replacement therapy (HRT) use and risk of lung cancer incidence and death. The first reports of a possible link between HRT use and increased lung cancer risk were suggested in the late 1980s by Wu *et al.* in a small population-based case–control study of 672 women [24] and in a larger population study of 23,244 women by Adami *et al.* [25]. Another small case–control study conducted by Taioli and Wynder also provided evidence of an increase in lung cancer risk in women using HRT and demonstrated a positive interaction between HRT, smoking and lung cancer development [26]. Ganti and colleagues performed a retrospective chart review of 498 women with lung cancer and have shown that overall survival (OS) was significantly increased in lung cancer patients who never took HRT compared with those who received HRT and the effect was strongest in women who smoked [27]. However, some studies do not support an association between HRT use and increased lung cancer risk and this still remains a controversial subject [28–33]. Differences in these studies may be related to type of HRT used, duration of use or time of use relative to lung tumor development.

A small number of recent studies examined risk by HRT type including estrogen only or estrogen plus progestin (E+P). The Women's Health Initiative study which was a randomized, double-blind, placebo-controlled trial of E+P and included over 16,000 healthy post-menopausal women showed that E+P use significantly increased the number of lung cancer deaths compared with women in the placebo arm [34]. Interestingly, E+P use in this

study did not lead to an increased incidence of lung cancer suggesting that HRT led to more aggressive NSCLC with a poorer prognosis. However, in a separate study conducted by the Women's Health Initiative, which examined over 10,000 post-menopausal women who had a previous hysterectomy and were randomized to receive either estrogen alone or placebo, the use of estrogen alone did not increase lung cancer incidence or death [35]. This relationship was also examined in the Vitamins and Lifestyle Study. In this large prospective population-based cohort of more than 36,000 women, E+P HRT use increased lung cancer incidence in a duration-dependent manner, with the highest risk observed with >10 years of E + P use [36]. Although mortality was not analyzed in this particular study, a positive association between E + P use and advanced stage at diagnosis was observed. Additionally, there was no association with estrogen-only HRT use and lung cancer incidence in this study. The biological mechanisms underlying these population-based observations can be partially explained by estrogen signaling pathways identified in the lung. These observations are most likely caused by highly complex interactions between multiple factors including hormonal, genetic and metabolic components and requires further investigation. HRT use is now recommended only for short-term treatment in postmenopausal women so lung cancer risk through exogenous hormone use may decline in the future.

Antiestrogens protect against lung cancer mortality

The critical role of estrogen in lung carcinogenesis is further reinforced by the observation that antiestrogen usage is associated with decreased lung cancer mortality risk. Antiestrogen use was evaluated in 6655 women diagnosed with breast cancer between 1980 and 2003 in the Geneva Cancer Registry [37]. Interestingly, a fivefold reduction of lung cancer mortality was observed in the breast cancer patients who received antiestrogen therapy compared with expected rates in the general population. Lung cancer incidence was not associated with antiestrogen therapy in this study. An additional study utilizing data from the Manitoba Cancer Registry confirms these results [38]. In this study, 2320 women with NSCLC were included in the study and antiestrogen use was found to significantly decrease lung cancer mortality in women who received antiestrogens both prior to and after a lung cancer diagnosis [38]. Clearly, the role of antiestrogen therapy on lung cancer outcome should be further explored.

Critical mediators of the estrogen signaling pathway are expressed in human lung cancer & correlate with lung cancer survival

Estrogen receptors in lung carcinomas

Cellular responses to estrogen are mediated by the estrogen receptors, ER α and ER β which are encoded by separate genes and have differential tissue distributions. It is now evident that ER β is the dominant ER in most primary specimens of human NSCLCs from both men and women [39–45]. This is in contrast to the predominant expression of ER α protein in breast cancer [46]. ER α expression shows great variability in its expression frequency among the reported studies [40–42,44,45,47]. ER α is generally found in lung tumors at a lower frequency of expression compared with ER β and has been found to be highly correlated with *EGFR* mutation [45,48]. Both ER α and ER β have been demonstrated to be expressed at higher levels in tumor tissue compared with matched normal lung [40]. ER β

also plays a role in normal lung development as shown by numerous lung abnormalities in the ER β knockout mouse including decreased alveoli, reduction in key regulators of surfactant homeostasis, alveolar collapse and extracellular matrix alterations [49,50]. Both ER α and ER β protein are localized to both the nucleus and the cytoplasm in lung tumors and staining in both cellular compartments is important [22,40,51]. Nuclear ER β expression was found to be a positive prognostic marker in some studies but only in certain subsets of patients [44,52]. Conversely, cytoplasmic ER β -1 expression was found to be a strong negative prognostic indicator for lung cancer in both males and females [40]. In a separate study, nuclear ER β -1 also correlated with poor survival but only in the metastatic setting [53]. By contrast, other ER β isoforms, such as ER β -2 and ER β -5 have been linked to better lung cancer outcome [54]. Finally, ER α expression in relation to lung cancer survival has shown either no effect or has been correlated with poor prognosis [40,43,44]. Clearly, both cytoplasmic and nuclear ERs are important and cellular localization should be taken into account when analyzing lung tumor ER expression.

Estrogen synthesis in lung carcinomas

Estrogen can also be locally synthesized in lung tumors [46,55] and the aromatase enzyme which catalyzes the conversion of androgens to estrone and estradiol is highly expressed in lung tumor tissue [40,56,57]. Estradiol levels correlated with aromatase expression and estradiol concentrations were significantly higher in lung tumors compared with normal lung tissue [46]. In addition, aromatase expression was shown to be higher in metastases compared with matched primary lung tumors [58]. A high frequency of coexpression of aromatase and ER β has been reported [59]. Furthermore, high aromatase tumor expression correlates with worse survival in a cohort of postmenopausal women with early stage lung cancer [56]. In addition, when combined with ER β , aromatase was a strong predictor of clinical outcome in both men and women [60]. Similarly, in a separate report that included both men and women with all stages of lung cancer, aromatase expression was not predictive unless combined with other hormonal markers such as ER β and progesterone receptor (PR) [40].

Other steroid hormones related to the estrogen signaling pathway, such as PR, have also been evaluated in lung tumors. Reported PR expression in lung tumors is also quite variable [40,41,45,47,59,61–63]. While some reports show little to no PR expression in lung tumors [41,59,63], others have suggested that high PR expression is strong protective factor for lung cancer [47]. Interestingly, PR expression was found to be lower in tumor tissue compared with matched normal lung from the same patient [40] despite the fact that enzymes capable of synthesizing progesterone are detectable in many NSCLC tumors [47]. A small, but significant, subset of early stage-resected patient samples stain strongly for cytoplasmic ER β without any or low PR expression and have an extremely poor prognosis [40]. This likely represents a subset of tumors in which nongenomic signaling through the ER pathway predominates as explained in the following section.

Preclinical data demonstrates that estrogen is a driver of lung cancer

Estrogen induces cell proliferation of NSCLC cells *in vitro* [22,39], in human tumor xenografts [39] and in animal models of lung cancer [64]. Estrogen exerts its effects through

both genomic and nongenomic mechanisms in the lung as shown in Figure 2. Genomic estrogen effects include modulation of genes in NSCLC cell lines that are important for inducing cell proliferation, such as c-myc and cyclin D1 as well as the canonical ER responsive gene, *PR* [65]. Estrogen also induces transcription through ERE and AP-1 sites and signaling through these promoter elements was shown to occur primarily through ER β in NSCLC cells [39,66]. ER β was also responsible for lung tumor xenograft growth in mice [66]. In addition, the pure antiestrogen fulvestrant, as well as the aromatase inhibitors exemestane and anastrozole, inhibit proliferation in NSCLC cell lines and in lung tumor xenograft models in immunocompromised mice [39,55,58].

Estrogen has also been demonstrated to interact with several growth factor signaling pathways, including EGFR, IGFR-1 and VEGF receptor signaling pathways (Figure 2). These nongenomic effects occur independently of transcription and translation and are thought to be mediated by a cytoplasmic or membrane-associated population of ERs. Nongenomic signaling involving EGFR activation by ERs is a predominant signaling pathway in NSCLC and may portend a poor prognosis [21,22]. Estrogen induces rapid signaling through the EGFR and MAPK pathways within minutes [21]. Furthermore, because of this crosstalk, effectiveness of EGFR TKIs can be enhanced by antiestrogens or aromatase inhibitors [21,22,67–69]. Similar results are observed when antiestrogens are combined with IGF receptor-1 inhibitors [70], as well as with the multitargeted TKI, vandetinib [58,71].

Recently, these antiestrogen therapies were tested in an animal model of lung cancer prevention [72]. One protocol assessed whether anastrozole and fulvestrant inhibit the initiation of precancerous changes while a second protocol determined whether these treatments could halt the development of precancerous lesions into full-blown lung tumors. Both drugs showed fewer tobacco carcinogen-induced lung tumors compared with placebo, however the combination of the two drugs resulted in maximum anti-tumor effects in both protocols suggesting that these drugs may have application for lung cancer prevention. Interestingly, in this animal model, aromatase expression in lung tumors was confined almost exclusively to infiltrating inflammatory cells. An important source of estrogen synthesis may be inflammatory cells that infiltrate the lung early in the carcinogenic process. This pre-clinical evidence combined with population data strongly support targeting the estrogen pathway therapeutically for lung cancer (Figure 1).

Antiestrogen therapy for treatment and prevention of ER/PR positive breast cancer

The development of antiestrogen therapy for hormone positive (ER⁺, PR⁺ or ER⁺ or PR⁺) breast cancer was directly responsible for a 28% decrease in the breast cancer mortality rate in the USA alone since the late 1980s [2]. Therefore, first-line hormonal therapy in both the adjuvant and advanced setting has become the standard of care [73]. The first antiestrogen therapy was primarily surgical and the first case series of oophorectomies for ovarian suppression was reported in 1896 [74]. In the late 1970s, the first effective medical antiestrogen therapy, selective estrogen receptor modulators (SERMs), were tested and approved in the metastatic setting. Shortly after this, the first biomarker for tamoxifen

response, namely tumor ER and PR expression was described and has become one of the gold standards for selecting patients for antiestrogen treatment [75]. Since this time, it has been shown that tamoxifen exerts many other anti-tumor biological effects that are independent of ER status [76].

Futhermore, a series of large randomized Phase III studies have demonstrated the efficacy of tamoxifen in both the metastatic and adjuvant setting for both pre- and post-menopausal women [73]. In the adjuvant and advanced setting in post-menopausal women, the aromatase inhibitors (anastrozole, letrozole and exemestane) have been demonstrated to be superior to tamoxifen and have become standard of care for first line therapy [73]. Aromatase inhibitors are only useful in the postmenopausal setting since they work by inhibiting the conversion of androgens to estrogen in the peripheral tissues (Figure 2) [77]. Finally, a third class of antiestrogen therapy (fulvestrant) is also approved in the second-line setting for advanced breast cancer. Fulvestrant works through a distinct mode of action by directly binding the ER and leading to its degradation (Figure 2). Clinical use and interest in this compound has recently increased since it was found to be more efficacious at a higher dose than originally approved for by the US FDA [78].

In addition to a role of antiestrogens in treatment of hormone positive breast cancer, a role for primary prevention of breast cancer was established in a series of Phase III trials (NSABP P-1 and IBIS-1) for tamoxifen in high risk women [79,80]. In the first and larger of the two trials (NSABP P-1), 13,388 women at high risk for breast cancer were randomized to 5 years of tamoxifen versus placebo and a 49% risk reduction in invasive breast cancer was observed. A similar result was seen in the smaller IBIS-1 trial and the benefit of therapy persisted for up to 5 years after stopping therapy [80]. The success of antiestrogen therapy in breast cancer can serve as a model for the development of antiestrogen therapy in the treatment and possible prevention of NSCLC.

Antiestrogen therapy in NSCLC: results of early phase clinical trials

The investigation of the role of antiestrogen therapy has been an evolving and ongoing process for over a decade (Figure 1) and has cumulated in two early phase clinical trials in NSCLC with several more in the planning stage. The rationale for these early trials was based on preclinical data suggesting that dual inhibition of the ER and EGFR pathways leads to enhanced anti-tumor activity. In 2009, the results of the first trial of antiestrogen therapy in NSCLC were published and demonstrated safety and potential efficacy [3]. This Phase I trial looked at the combination of fulvestrant and the EGFR TKI gefitinib in postmenopausal women with advanced NSCLC and allowed for multiple prior lines of therapy and any histology of NSCLC. Twenty-two patients were enrolled and 20 patients were evaluable for response. Interestingly, three confirmed partial responses were observed (response rate of 15%; 95% CI: 5–36%). Of note, one of these patients was found to have a TKI-sensitizing *EGFR* mutation. The median progression-free survival (PFS), 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. Remarkably, a survival analysis based on ER β nuclear staining revealed a striking, albeit statistically insignificant, difference in OS. In patients whose tumors exhibited at least 60% ER β nuclear IHC staining there was an OS of 65.5

weeks versus only 21 weeks in the patients with less than 60% ER β positivity. In addition, the combination was well tolerated.

These promising results lead to a subsequent randomized Phase II study using the combination of erlotinib and fulvestrant versus erlotinib alone in the second-line advanced setting [4]. In this trial, patients were randomized to the EGFR TKI erlotinib alone (ERT arm) versus the combination of ERT and fulvestrant (ERT+F arm). ERT was substituted for gefitinib since gefitinib was no longer available in the USA. In addition, patients received a clinically more effective dose of F (500 mg vs previous 250 mg dose in the Phase I). In this trial, men and women with advanced NSCLC in the second-line setting were randomized 2:1 to receive ERT+F versus ERT. A total of 106 patients were randomized and 100 patients were evaluable. The combination was well tolerated and adverse events well balanced between arms. In the entire patient population, ERT+F and ERT (response rate: 23.6 vs 14.8%, respectively; $p = 0.35$), PFS (1.9 vs 1.8 months, respectively; hazard ratio [HR]: 0.85, 95% CI: 0.55–1.33) and OS (9.4 vs 5.7 months, respectively; HR: 0.96; 95% CI: 0.6–1.55) were similar between arms. This trial was conducted prior to routine molecular testing being performed for the *EGFR* TKI-sensitizing mutations and a retrospect analysis was performed of the 69 patients whose *EGFR* mutation status was available. As expected, *EGFR* mutations strongly predicted best response, PFS and OS ($p = 0.0002$ for each). However, among the 52 patients with *EGFR* wild-type (WT) tumors, three partial responses were seen with ERT+F versus none for E alone and clinical benefit rate (clinical benefit rate [response rate + stable disease]; 54.8 vs 8.3%, $p = 0.0056$) was significantly higher among patients with WT tumors treated with ERT+F. Finally trends were observed in favor of ERT +F in PFS (2.0 vs 1.6 months; HR: 0.56; 95% CI: 0.29–1.07) and OS (7.4 vs 5.9 months; HR: 0.69; 95% CI: 0.36–1.31). Although these results appear promising, the preplanned evaluation of tumor tissue biomarkers (including ER α , ER β and aromatase) and serum biomarkers (estrogen levels and EGFR ligands) will be critical to determine which NSCLC subpopulations are most likely to benefit from antiestrogens. Of note, only ER β expression was examined as a biomarker in the Phase I trial and it remains unanswered whether markers from the ER and/or EGFR pathways will predict response in the *EGFR* WT population.

In addition to the above trials looking at the combination of ER blockade and EGFR inhibition, the efficacy of the aromatase inhibitors is currently being examined in ongoing or planned trials. In the metastatic setting, exemestane is being examined in combination with chemotherapy in the first-line setting (NCT01664754) [101]. Furthermore, a Phase II trial examining the potential role of exemestane in the adjuvant setting in postmenopausal women is currently in the planning stages [Burns TF, Stabile LP, Pers. Comm.].

Conclusion & future perspective

Hormonal therapies targeting the estrogen pathway are well-established in the breast cancer setting for both therapeutic treatment as well as prevention. As these drugs have been thoroughly studied in breast cancer, the toxicity profile is well-established and they are readily accessible. Over the last decade, the significance of the estrogen signaling pathway in lung tumorigenesis has been established and early phase clinical trials have suggested that

the use of antiestrogens is extremely promising in lung cancer. Further well-designed clinical trials will be necessary before these drugs can move into clinical practice for lung cancer. It will be crucial to develop biomarkers of response in these trials so that the best candidates for antiestrogen and whether or not these agents should be combined with other targeted agents for maximum benefit can be determined. Since both men and women express ERs and aromatase, these treatments may benefit both male and female lung cancer patients. Finally, these drugs may eventually be used for primary prevention in patients with a high risk of developing lung cancer.

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Website

101. Exemestane, Pemetrexed Disodium, and Carboplatin in Treating Post-Menopausal Women With Stage IV Non-Small Cell Lung Cancer. <http://clinicaltrials.gov/show/NCT01664754>

Practice Points

- Estrogen signaling promotes lung tumorigenesis through both genomic and nongenomic signaling mechanisms.
- Hormone replacement therapy is associated with increased lung cancer mortality and incidence.
- The use of adjuvant antiestrogen therapy in breast cancer patients reduces the risk of lung cancer.
- Estrogen receptor- β is the predominant estrogen receptor isoform in lung cancer and cytoplasmic estrogen receptor- β is a poor prognostic marker for non-small-cell lung cancer.
- Preclinical studies reveal a critical role for estrogen signaling in promoting lung cancer proliferation; antiestrogen and aromatase inhibitor therapies are effective in non-small-cell lung cancer.
- Several clinical trials have recently been completed and others are underway to test endocrine therapies in combination with either targeted therapy or chemotherapy in advanced lung cancer patients.
- Positive results from advanced therapy trials suggest that these treatments should also be examined in earlier stages of lung cancer or in the prevention setting.
- Biomarker identification that predicts which lung cancer patients are the best candidates for hormonal therapy will be necessary to guide the design of future trials.
- Research on estrogen signaling in lung cancer is likely to benefit both men and women.

Figure 1. Overview of important milestones in the development of antiestrogen therapy in non-small-cell lung cancer over the last decade

ER: Estrogen receptor; NSCLC: Non-small-cell lung cancer.

Figure 2. Model of genomic and nongenomic estrogen signaling in non-small-cell lung cancer
 Estrogen action in non-small-cell lung cancer can occur through both genomic and nongenomic mechanisms in the lung. Nuclear ERs can be activated in a ligand-dependent manner by E2 binding to nuclear ERs at estrogen-responsive elements or AP-1 sites in the promoters of estrogen-regulated genes. Nongenomic estrogen action in the lungs occurs through rapid activation of EGFR in the cell membrane, which occurs through activation of Src and MMP cleavage of the EGFR proligands to activate EGFR and the ERK and Akt downstream signaling pathways. Both of these mechanisms of estrogen action in non-small-cell lung cancer led to cell proliferation and survival. Available strategies to target the estrogen signaling pathway for lung cancer treatment or prevention are shown in red and include: (A) inhibition of estrogen synthesis with aromatase inhibitors, such as anastrozole or exemestane; (B) downregulation of ERs using antiestrogens such as fulvestrant; and (C) targeting growth factor pathways that are activated by estrogens with agents such as gefitinib or erlotinib. These strategies can be used as single agents or in combination. E2: β -estradiol; EGFR: EGF receptor; ER: Estrogen receptor; HB: Heparin-binding; MMP: Matrix metalloproteinase.