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Cancer Prevention Research

Cotargeting Cyclin D1 Starts a New Chapter in Lung Cancer Prevention and Therapy

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Abstract

Lung cancer has limited effective therapy and no effective prevention. Cytotoxic chemotherapy has not improved when combined with the epidermal growth factor receptor (EGFR) inhibitor erlotinib (standard lung cancer therapy) or with the rexinoid bexarotene. Combining erlotinib and bexarotene, however, to cotarget cyclin D1 via the retinoid X receptor and EGFR was active preclinically in *KRAS*-driven lung cancer cells derived from transgenic mice and in two clinical studies in lung cancer (including wild-type *EGFR* tumors, with or without *KRAS* mutations), as reported in this issue of the journal by Dragnev and colleagues (beginning on page 818). These results, along with closely related clinical results of the BATTLE program, support the promise of this cotargeting approach for lung cancer prevention and therapy and of cyclin D1 as a predictive, personalizing marker for it. *Cancer Prev Res; 4(6); 779–82. ©2011 AACR.*

Introduction

Non–small cell lung cancer (NSCLC) is a heterogeneous tumor, which has been defined historically by histology (e.g., adenocarcinoma, squamous cell) and increasingly by the addition of molecular characteristics (1). Chemotherapy in general for this disease has been limited by toxicity and a lack of specificity and did not improve when combined with the epidermal growth factor receptor (EGFR) inhibitor erlotinib (2, 3) or with the rexinoid bexarotene (4, 5). Lung cancer chemoprevention has foundered heretofore for a variety of reasons, although very recent clinical data suggest promise in this setting (6). The future of lung cancer therapy is growing brighter in light of the promising, increasingly accepted concept that biomarker discoveries can guide the selection of patients for the most appropriate treatment. For example, this concept has been applied clinically in the setting of adenocarcinoma by targeting *EGFR* mutations with EGFR tyrosine kinase inhibitors (7, 8) and, more recently, by targeting echinoderm microtubule–associated protein-like 4–anaplastic lymphoma kinase (*EML4–ALK*) translocations with ALK inhibitors (9). Patients with tumors containing either of these alterations, however, constitute less than 20% of the NSCLC patient population. Therefore, effective therapies are an urgent unmet need for the other, greater than 80% of NSCLC patients, including those with wild-type *EGFR* adenocarcinoma, especially with *KRAS* mutations, or with squamous cell carcinomas.

The work reported by the Dmitrovsky group in this issue of the journal (10) is the culmination of more than a decade of prevention- and therapy-related preclinical and clinical studies in the lung that focused on targeting cyclin D1. On the basis of the rationale of dual targeting of cyclin D1 (via different mechanisms), these investigators tested (and now report the results of) preclinical and 2 clinical studies of the retinoid X receptor (RXR) agonist bexarotene combined with the EGFR tyrosine kinase inhibitor erlotinib. The key new preclinical findings show that cyclin D1 expression increases during progression from premalignant lesions to invasive and metastatic lung cancer in a cyclin E transgenic model [which was reviewed recently in this journal; ref. (11)] and that combined bexarotene and erlotinib repressed cyclin D1 and cell growth far more than did either agent alone in the cyclin E transgenic model and in *KRAS*-driven lung cancer cells derived from the aggressive *KRAS*/*p53* transgenic model. Key new clinical results include the effects of combination in reducing cyclin D1 expression (by $\geq$50% in four patients) and inducing 20% necrosis or greater in 4 patients (including 3 with *KRAS* mutations) in a short-term (median: 7 days), small-scale ($n = 10$) trial in early-stage NSCLC with wild-type *EGFR*. The second new clinical study, a phase II trial, considered together with this group's related earlier phase I trial (12), shows that the combination induced major clinical responses in advanced NSCLC with wild-type *EGFR* (with or without *KRAS* mutations).

These phase II and I results are provocative for several reasons. They show the activity of combination in the generally resistant population of patients with wild-type *EGFR* with or without *KRAS* mutations. They involve a relatively large overall number of NSCLC patients (phase II,

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$n = 42$; phase I, $n = 19$). Their context includes our group's trial of this combination within the Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) in virtually an identical patient population (13). The prior phase I trial produced 4 major objective radiographic responses in its 16 patients (all had wild-type *EGFR* tumors but *KRAS* mutations were not assessed). The new phase II trial (10) produced major responses in 3 patients (1 complete and 2 partial responses) and unusually long survivals. These 3 patients included 1 with an *EGFR*/*KRAS* wild-type tumor and 1 with a *KRAS* mutation (which, of note, do not coexist with *EGFR* mutations). The patient with the *KRAS*-mutant tumor was a male former smoker, had received 2 prior therapies, had a partial response, and survival of 665 days following treatment.

These clinical findings are consistent with and validate our own recent observations of the efficacy of combination in BATTLE (13), which comprised 4 separate trials. An important distinction between the Dmitrovsky group and our BATTLE phase II trials, however, is that only responders were sequenced in the former, whereas every patient in BATTLE was sequenced to personalize therapy. BATTLE conducted real-time examinations of the following biomarkers: *EGFR*, *KRAS*, and *BRAF* mutations by PCR; *EGFR* and cyclin D1 copy number by FISH; and the proteins VEGF, VEGF receptor 2 (VEGFR2), RXRα, β, γ, and cyclin D1 (all by immunohistochemistry), which are related to 4 molecular pathways (EGFR, KRAS/BRAF, VEGFR, and nuclear RXR/cyclin D1) frequently disrupted in NSCLC. On the basis of their biomarker profiles, 255 heavily pretreated patients were randomized (via a Bayesian adaptive design) to 1 of the 4 BATTLE trials targeting the pathways of interest as follows: erlotinib (targeting EGFR), vandetanib (targeting VEGFR), sorafenib (targeting *KRAS*), and bexarotene plus erlotinib (targeting cyclin D1). Random assignments to the erlotinib and bexarotene plus erlotinib trials were limited by exclusion of prior erlotinib (in about half of all 255 patients).

BATTLE randomized 37 patients to the bexarotene plus erlotinib trial. Key comparisons in 8 weeks disease control rate (DCR) between this combination trial (BE) and the BATTLE trial of erlotinib (E) as a single agent erlotinib ($n = 59$ patients), which is the Food and Drug Administration–approved standard of care in this setting, are as follows:

- Overall (the primary endpoint of both trials): 50% (BE) versus 34% (E).
- In patients with wild-type *EGFR* with or without *KRAS* mutations (22 such patients of 37 BE patients; 45 of 59 E patients): 45.5% (10 of 22 BE patients) versus 28.9% (13 of 45 E patients).
- In *KRAS*-mutant tumors: 60% (3 of 5 BE patients) versus 22% (2 of 9 E patients).
- In *EGFR*-mutant and FISH-positive (amplification and high polysomy) tumors: 55% (11 of 20 BE patients) versus 35% (6 of 17 E patients), the difference driven by the benefit in amplified *EGFR* tumors.

Although these DCR comparisons were not statistically significant, collectively they suggest that the combination improved outcome over standard erlotinib in patients with wild-type *EGFR* tumors and thus could help address the unmet medical need of these patients. Furthermore, these internally consistent trends were driven by cyclin D1 targeting.

Bexarotene plus erlotinib had highly statistically significant activity in patients with tumors that overexpressed cyclin D1. On the basis of previous results of the Dmitrovsky group, BATTLE preferentially assigned these patients in a hierarchical model under the Bayesian adaptive randomization scheme to the bexarotene plus erlotinib trial. Thus, the bexarotene plus erlotinib trial personalized therapy according to the prespecified hypothesis that the combination would be most effective in patients with high cyclin D1 tumors.

The 2 BATTLE winners were the trial of bexarotene plus erlotinib and the trial of sorafenib, producing similar DCRs overall and in certain molecularly defined subgroups. Eight-week DCRs overall were 58% (sorafenib) and 50% (bexarotene plus erlotinib [combination]); in wild-type EGFR tumors, DCRs were 64% (sorafenib) and 46% (combination); in *KRAS*-mutant tumors, DCRs were 61% (sorafenib) and 60% (combination); and in *EGFR*-mutant and FISH-positive tumors, DCRs were 39% (sorafenib) and 55% (combination). Despite the overall favorable comparison, only sorafenib moved forward to our group's BATTLE 2 (due to open accrual soon), which excluded bexarotene plus erlotinib because of substantially smaller patient numbers (vs. sorafenib) overall and with wild-type *EGFR* or *KRAS*-mutant tumors, making these BATTLE combination findings less robust. The combination and sorafenib results compare very favorably with those of erlotinib alone (e.g., 34% overall DCR) or vandetanib (e.g., 33% overall DCR). The combination could move forward now, however, in light of the cumulative weight of the data of our group and the Dmitrovsky group.

The body of work also supports future investigation of this combination for lung cancer prevention, with similar cyclin D1 considerations. Cyclin D1 overexpression marks an increased risk of upper aerodigestive tract premalignant lesions for progressing to cancer. Downregulation of cyclin D1 with the GG genotype in these lesions with a regimen based on the bexarotene relative 13-*cis*-retinoic acid (13cRA) was associated with cancer prevention (14, 15); the regimen did not downregulate overexpression of cyclin D1 with the AG or AA genotype [the A allele preferentially produces an altered transcript that resists retinoic acid degradation; ref. (16)] or prevent cancer in patients with such lesions. The prevalence of the GG genotype in cyclin D1 overexpression seems to be about 30%. Dual targeting of cyclin D1 with bexarotene and erlotinib potentially will overcome the retinoic acid/rexinoid limitation in patients with this common polymorphism because erlotinib can

transcriptionally repress cyclin D1 expression (17). Cyclin D1 is a very relevant prevention target, with frequent overexpression, in premalignant lung carcinogenesis, as shown, for example, by preclinical and clinical results of the Dmitrovsky group (10, 18). It will be important to establish whether high cyclin D1 is a risk and predictive (of bexarotene plus erlotinib efficacy) marker in the lung cancer prevention setting, paralleling the therapy setting.

An important finding of the Dmitrovsky group's phase II trial was a significant survival benefit associated with bexarotene-induced hypertriglyceridemia ($P = 0.001$), consistent with prior studies of bexarotene alone or combined with chemotherapy in NSCLC (4, 5, 19). Hypertriglyceridemia generally developed within 2 to 4 weeks in all these studies, and, long known in association with retinoids such as 13cRA (20), it does not seem to have adverse clinical effects. The rate of hypertriglyceridemia is substantially higher with bexarotene than with 13cRA, and monitoring the development of hypertriglyceridemia potentially could be an early indication of efficacy and thus a personalizing factor for prevention of NSCLC with bexarotene plus erlotinib. The same consideration applies to the generally far better known rash associated with an improved benefit on erlotinib (21). Further advantages of rexinoids (vs. retinoic acid derivatives) for NSCLC prevention are in bypassing the major retinoic acid target retinoic acid receptor β, which is frequently silenced in lung carcinogenesis (22), and in producing far less skin toxicity.

In summary, the collective studies of bexarotene and erlotinib highlighted in this perspective, both preclinical and clinical, send a signal of the promise of this combination for lung cancer therapy and prevention. The combination had preclinical activity in aggressive *KRAS*-driven lung cancer cells derived from transgenic mice. The clinical window-of-opportunity study indicated biological activity in classically resistant patients with wild-type *EGFR* tumors (including patients with *KRAS* mutations). This signal is amplified by the Dmitrovsky group's earlier phase I and current phase II trials, totaling 61 patients with 7 major responses, 6 in wild-type *EGFR* tumors with or without *KRAS* mutations. The Dmitrovsky group phase II data suggest that hypertriglyceridemia predicts improved survival. Completely independently, our group further amplified the signal by showing an improved 8-week DCR (vs. standard erlotinib) overall and in wild-type *EGFR* tumors. Finally, the BATTLE result showing that high cyclin D1 expression predicted benefit, as hypothesized, personalizes the application of the combination. This body of work builds a case for future studies of bexarotene plus erlotinib in the unmet medical need population of patients, with wild-type *EGFR* tumors, with or without *KRAS* mutations, and supports the use of cyclin D1 levels to stratify or select patients for NSCLC therapy or prevention with the combination. Indeed, our future BATTLE plans include a trial of the combination in preventing new lung cancers in the adjuvant setting (23).

Disclosure of Potential Conflicts of Interest

E. Kim is a consultant/Advisory Board member of Genentech, Boehringer Ingerheim, Tragera, and Bayer.

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