

Altered Histology Provides a Positive Clinical Signal in the Bronchial Epithelium

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Abstract

The history of lung cancer chemoprevention trials has been uniformly disappointing in that the large phase III studies showed no effect or harm in actively smoking participants, and smaller phase II studies have also been negative. In this issue of the journal (beginning on page 793), Keith and colleagues report their randomized, placebo-controlled trial of the oral prostacyclin analogue iloprost, the first trial to show an improvement in bronchial histology (i.e., regression), which occurred in former, but not current, smokers with sputum atypia. This Perspective discusses the strength of the clinical signal provided by this observation and its implications for further drug development. *Cancer Prev Res*; 4(6); 775–8. ©2011 AACR.

Introduction

One of the main challenges in the development of drugs to prevent cancer is the difficulty in identifying efficacy in early-phase clinical trials. As with any drug development process, the decision to proceed to phase III clinical trials needs to be based on a thorough evaluation of the evidence suggesting the potential to prevent cancer and on an understanding of the toxicity profile of the intervention. Evidence of efficacy is derived from knowledge of the mechanisms (of carcinogenesis as well as of the action of the intervention), preclinical data, epidemiologic studies, and clinical trials (1). Even though clinical trials of specific interventions in actual people should theoretically provide the most relevant data to inform this decision-making process, the lack of validated surrogate endpoints has limited the ability of phase II prevention trials to predict phase III cancer incidence outcomes. Whereas phase II cancer treatment trials frequently use tumor measurements to assess efficacy, no equivalent measure of efficacy exists to serve as the primary endpoint for phase II cancer prevention trials. As a result, phase II cancer prevention trials generally assess multiple biomarkers, including premalignant histologic lesions, pharmacodynamic endpoints, proliferation indices, and other attributes of the process of carcinogenesis, to determine whether an intervention is active (2). The sufficient causal relationship to carcinogenesis to be an

adequate surrogate for cancer incidence and thus a useful target for chemopreventive drug development is established for some premalignant histologic lesions (otherwise known as intraepithelial neoplasias or IEN) and has been suggested for others (3).

In this issue of the journal, Keith and colleagues report their randomized controlled trial of the oral prostacyclin analogue iloprost in high-risk current and former smokers with sputum atypia, finding a positive effect on bronchial histology in the airways of former smokers (4). What do these results mean and how do we move forward with drug development based on this report? This Perspective will discuss the issues raised by using bronchial histology as an endpoint in lung chemoprevention clinical trials.

Bronchial Histologic Abnormalities Linked to Lung Cancer

The link between premalignancy and the subsequent development of cancer from that premalignancy is well established for some organ systems, but not for others. Removal of premalignant lesions is the standard of care and has been shown to decrease cancer incidence and mortality in the case of colorectal polyps and cervical IEN, with colonoscopies and Pap smears being part of routine health maintenance in high-resource countries such as the United States (3). In the case of oral premalignancy, however, only 60% of subsequent cancers arise at the site of the original oral leukoplakia, consistent with the idea that carcinogen exposure to the entire epithelial field results in multiple independent foci giving rise to independent cancers, or field carcinogenesis (5). The link between a discrete premalignant histologic bronchial abnormality and the development of subsequent lung cancer arising from that site is less well understood and is more difficult to study.

Ever since the autopsy studies by Auerbach and colleagues showing multiple histologic abnormalities in non-cancerous bronchial epithelium of smokers with and

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doi: 10.1158/1940-6207.CAPR-11-0214

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without lung cancer and the description by Saccomanno and colleagues of progressive sputum abnormalities preceding the development of lung cancer, it has been accepted that the development of squamous lung cancer proceeds in an orderly fashion through increasing grades of histologic abnormalities culminating in invasive carcinoma (6, 7). It has been much more difficult for a variety of reasons to show that lung cancers arise from any given premalignant lesion. First, the lung is an internal organ whose entire epithelial surface is not accessible to visualization and cell sampling in the manner that the entire colon, for example, can be studied via colonoscopy. Bronchoscopy only visualizes the central airways and thus is uninformative about the peripheral lung where adenocarcinomas, the most common form of lung cancer in smokers and nonsmokers alike, arise. Although the development of autofluorescence bronchoscopy has enhanced the detection of premalignant lesions in the central bronchial epithelium, this is more true of moderate/severe dysplasias and carcinoma *in situ* than it is of the earlier stages of premalignancy (8, 9). Therefore, the entire at-risk epithelium is not well visualized in the lung, and cancers are likely to arise from nonvisualized areas. Second, bronchoscopy-identified lesions are generally at least partially excised during biopsy. Several chemoprevention trials where sequential biopsies were conducted found per-subject response rates ranging from 28% to 30% versus per-lesion response rates ranging from 14% to 48% (depending on how the response rate was defined) in the placebo arms (4, 10, 11). Last, in addition to full lesion removal, biopsy causes tissue injury and subsequent tissue repair. It is not known how these factors affect the persistence, regression, or progression of premalignant lesions.

The incidences of the different degrees of bronchial dysplasia in heavy smokers are 44% (mild), 13% (moderate), and 6% (severe; ref. 9). Although studies of the natural history of these lesions are complicated by the removal of some lesions during sequential bronchoscopies with biopsies (discussed above), these studies suggest that about 3.5% of low or moderate dysplasias progress to severe dysplasia, 37% of severe dysplasias remain or progress, and about 50% of carcinomas *in situ* progress to invasive cancer in 2 to 3 years (reviewed in refs. 2, 9). Ishizumi and colleagues described their experience in the British Columbia Lung Health Study, where 2,154 heavy smokers with a 20-plus pack-years smoking history were screened with autofluorescence bronchoscopy prior to enrollment in chemoprevention trials (9). Lung cancer developed in 101 of these subjects, with a mean follow-up of 9.2 years. Progression to carcinoma *in situ* or invasive cancer in previously biopsied sites was 5.6% for initial diagnoses of severe dysplasia and 4.9% for initial moderate dysplasia. The progression rates were 0.8% for initial biopsies showing metaplasia/mild dysplasia and 0.9% for initial normal/hyperplasia. Furthermore, more cancers developed from sites other than the initially biopsied sites, although it must be pointed out that lack of a biopsy does not preclude the presence of a premalignant lesion at those sites.

Taken together, these data suggest that bronchial dysplasia is a marker of risk for the development of lung cancer somewhere in the lung, although not necessarily in the site of the index lesion. Increasing histologic abnormality is associated with increasing lung cancer risk. Nevertheless, given the inability to visualize the entire at-risk lung and the partial-to-total extirpation of at least some of the lesions during biopsies, it cannot be concluded that the low rate of progression from varying types of dysplasia to invasive cancer is evidence against the stepwise histologic progression sequence of squamous carcinogenesis proposed by Auerbach and Saccomanno. Instead, these data argue against a screening approach that removes premalignant lesions, which is very effective in preventing colorectal and cervical cancer, and rather suggest that systemic treatment to prevent the progression of nonvisualized premalignant lung lesions is more likely to be effective.

Definitions of Response and Clinical Benefit in Bronchial Dysplasia Trials

One of the more difficult issues complicating the development of intermediate endpoints is the need to be able to link clinical benefit to a quantitative measure of marker modulation. Before asking how much marker modulation is enough to produce a biologically significant effect, one needs to define response. Not surprisingly, there are no standard definitions of histologic response with respect to bronchial dysplasia. One of the strengths of the iloprost trial by Keith and colleagues is its rigorous method of assessing histologic response in a double-blind, placebo-controlled, multicenter trial with central/blinded pathology review. The investigators conducted multiple assessments of histologic response, which ranged from the average score (the primary endpoint, defined as the average of World Health Organization (WHO) grade classifications assigned to all biopsies; ref. 12) to the worst score and included the dysplasia index, all of which were consistent. Analyses conducted on a per-lesion basis and on a per-subject basis were consistent as well. The weakness, however, consists of defining a response as an improvement of as little as one grade level. The intraobserver reproducibility was 86% for diagnoses within 1 grade on the WHO scale and the interobserver reliability was 84%, both of which show a reassuringly high rate of consistency in the pathologic reviews by highly specialized pulmonary pathologists. However, in the context of a definition of response that was as small as a one-grade change in histology (e.g., from mild dysplasia to metaplasia) and an average improvement in iloprost-treated former smokers that was also approximately 1 grade (even though lesser improvement was seen in the placebo arm and in iloprost-treated current smokers), it is hard to interpret the biological significance of the effect. Previous studies by Lam and colleagues used the more stringent response criterion of a change in at least 2 grade levels to qualify as a response or progression (10). The reason for the more stringent criterion was the belief that only a very strong

signal in a phase II trial would be sufficient evidence to support a subsequent phase III trial.

Another problematic issue is the nature of the lesion being modulated by the intervention. In the iloprost trial, baseline histology was an average grade of 2.1 in former smokers and 3.0 for current smokers, corresponding to hyperplasia and metaplasia, respectively, as defined by WHO classification criteria for premalignant bronchial lesions (12). These findings suggest that the majority of biopsies had normal or early premalignant changes only, although 74% of participants reportedly had at least 1 area of mild dysplasia or a worse histology. Hyperplasia and metaplasia are responses to a variety of pulmonary insults and are easily reversible on cessation of these insults such as smoking cessation (13). One would like to know whether iloprost had any effect on advanced premalignant lesions, which are more likely to be persistent and are more closely associated with subsequent development of cancer, as discussed previously. Given the belief that lung carcinogenesis occurs over many years and that damage due to ongoing tobacco use results in premalignant lesions in varying stages of progression, an ideal chemopreventive agent would need to be effective at least during the late stages of carcinogenesis, if not throughout the entire process. Unfortunately, it is not feasible to conduct clinical trials exclusively in such a high-risk population because of the low prevalence of advanced lesions such as severe dysplasia in biopsied smokers, as discussed earlier. This issue is not trivial, however, as interventions may have different effects during varying stages of carcinogenesis.

The most difficult issue, however, is to determine how much regression of abnormal histology indicates effectiveness. Statistical significance is a prerequisite, but it is hard to understand how statistical significance translates to clinical benefit unless the effect is clearly very large. One could theorize that per-subject responses would be more clinically significant than per-lesion responses, as progression to invasive cancer in just 1 premalignant lesion would completely negate any benefit from the complete regression of multiple other lesions. Response Evaluation Criteria in Solid Tumors (RECIST)-type criteria, such as a 30% reduction in a biomarker (or in the number of bronchial dysplasias), could be applied, but there is no clear biological basis for choosing such a cutoff point for evaluating premalignancies. One must keep in mind that even in phase II cancer treatment trials, tumor shrinkage does not necessarily equate to enhanced survival and thus response rate is not a validated surrogate for real clinical benefit in all tumor types (14).

Another point to consider is that, given the use of non-cytotoxic agents in prevention, is it realistic to be looking for lesion regression or could the lack of progression be a more important indicator of efficacy? Certainly in the era of molecular-targeted therapy of cancer, survival benefits are being seen in the absence of substantial phase II response rates based on tumor shrinkage, and the converse is also true, with phase III failures for therapies that had shown significant responses in phase II testing (14). In the case of

lung chemoprevention trials, only one previously reported phase II trial, which tested anethole dithiolthione, found a statistically significant reduction, albeit a small one, in the progression rate for the intervention arm, although no regression of bronchial dysplasia was noted (15), in contrast to regression in the iloprost trial.

In view of the caveats about the interpretation of response in bronchial dysplasia trials, several conclusions should be drawn. Criteria for response should be stringent to ensure that a reported change truly reflects a difference in biology. Multiple aspects of the response rate need to be assessed for each trial. The rates of regression, lesion stability, and progression to higher grades of dysplasia all need to be clearly reported. Response should be analyzed on a per-lesion as well as per-subject basis. Furthermore, other biomarkers of drug effect on the process of carcinogenesis should be assessed in addition to histology to complement the histologic response data. Given the experience with cancer treatment trials, where response rates tend to decrease as one moves from phase II trials conducted in selected treatment centers to phase III trials in a larger community setting, only a large, robust effect in the prevention phase II setting is likely to translate to a phase III cancer incidence reduction.

Implications of the Iloprost Trial

How should we interpret the iloprost trial reported by Keith and colleagues (4)? Bronchial histology remains one of the best available indicators of cancer risk that can be measured sequentially over time. Because higher grades of dysplasia appear to be less reversible and more predictive of subsequent lung cancer than are earlier histologic lesions such as metaplasia, regression of the higher-grade lesions likely is more informative about cancer preventive effects of an agent. Given the dynamic nature of bronchial histologic lesions with a "spontaneous" regression rate that has been documented by serial biopsies, it is essential to conduct clinical trials in a blinded fashion with concurrent placebo controls as was done in the present study.

It must be emphasized that bronchial dysplasia is a precursor for squamous carcinogenesis (arising in the central lung) and is not necessarily informative about prevention of cancer arising in the peripheral lung. Although iloprost has been shown to have preventive activity in mouse models of peripheral lung adenomas, comparable human data (e.g., effects of iloprost on computed tomography-detected indeterminate nodules that may be precursors to peripheral adenocarcinomas) are not yet available (16). As we move forward with lung chemoprevention trials such as this one, assessment of the effect of agent on bronchial histology remains a necessary component of the drug development process, even as our understanding of the meaning of such trial results continues to evolve. Combining these results with an assessment of the effects of agent on peripheral lung carcinogenesis and molecular processes involved in tumor progression will

provide the robust data needed to adequately inform drug development decisions.

It is of interest that histologic effect of iloprost was only seen in former smokers and not in current smokers in the present trial, underscoring the importance of studying these 2 populations separately. Two recent studies have documented a biomarker response (a reduction in Ki-67 proliferation index) in former smokers treated with celecoxib, although neither study was designed to detect an effect on bronchial histology (17, 18). One of these trials also found a decrease in Ki-67 in current smokers, albeit of a lesser magnitude than in former smokers. Because premalignant histology is more closely associated with progression to lung cancer than is proliferation, the iloprost trial is more convincing about the potential of drug to prevent cancer but still must be interpreted with caution because of its small number of former smokers, less-stringent response criteria allowing a one-grade difference in histology, and responses that seem to have been driven more by effects on early premalignancy rather than on severe dysplasia. Strengths of the positive iloprost signal, however, include its occurrence in a randomized, placebo-controlled trial with blinded pathology review of its histology endpoint measured by average and worst WHO score and dysplasia index, all producing consistent results. These results support a follow-on randomized, controlled trial in former smokers, which, if consistent with the present trial, would make a strong case for advancing this agent to a phase III trial.

References

- Szabo E. Selecting targets for cancer prevention: where do we go from here? *Nat Rev Cancer* 2006;6:867–74.
- Kelloff GJ, Lippman SM, Dannenberg AJ, Sigman CC, Pearce HL, Reid BJ, et al. Progress in chemoprevention drug development: the promise of molecular biomarkers for prevention of intraepithelial neoplasia (IEN) and cancer (AACR Cancer Prevention Task Force). *Clin Cancer Res* 2006;12:3661–97.
- O’Shaughnessy JA, Kelloff GJ, Gordon GB, Dannenberg AJ, Hong WK, et al. Treatment and prevention of intraepithelial neoplasia: an important target for accelerated new agent development. *Clin Cancer Res* 2002;8:314–46.
- Keith RL, Blatchford PJ, Kittelson J, Minna JD, Kelly K, Massion PP, et al. Oral iloprost improves endobronchial dysplasia in former smokers. *Cancer Prev Res* 2011;4:793–802.
- Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, et al. Predicting cancer development in oral leukoplakia: ten years of translational research. *Clin Cancer Res* 2000;6:1702–10.
- Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in the bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. *N Eng J Med* 1961;265:253–67.
- Saccomanno G, Archer VE, Auerbach O, Saunders RP, Brennan LM. Development of carcinoma of the lung as reflected in exfoliated cells. *Cancer* 1974;33:256–70.
- Lam S, MacAulay C, Hung J, LeRiche J, Profio AE, Palcic B. Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscope device. *J Thorac Cardiovasc Surg* 1993;105:1035–40.
- Ishizumi T, McWilliams A, MacAulay C, Gazdar A, Lam S. Natural history of bronchial preinvasive lesions. *Cancer Metastasis Res* 2010;29:5–14.
- Lam S, leRiche JC, McWilliams A, MacAuley C, Dyachkova J, Szabo E, et al. A randomized phase IIb trial of pulmicort turbuhaler (budesonide) in persons with dysplasia of the bronchial epithelium. *Clin Cancer Res* 2004;10:6502–11.
- Lam S, McWilliams A, leRiche J, MacAulay C, Wattenberg L, Szabo E. A phase I study of myo-inositol for lung cancer chemoprevention. *Cancer Epidemiol Biomarkers Prev* 2006;15:1526–31.
- Nicholson AG, Perry LJ, Cury PM, Jackson P, McCormick CM, Corrin B, et al. Reproducibility of the WHO/IASLC grading system for pre-invasive squamous lesions of the bronchus: a study of inter-observer and intra-observer variation. *Histopathology* 2001;38:202–8.
- Lee JS, Lippman SM, Benner SE, Lee JJ, Ro JY, et al. Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial metaplasia. *J Clin Oncol* 1994;12:937–45.
- Bradbury P, Seymour L. Tumor shrinkage and objective response rates: gold standard for oncology efficacy screening trials, or an outdated end point? *Cancer J* 2009;15:354–60.
- Lam S, MacAulay C, le Riche JC, Dyachkova Y, et al. A randomized phase IIb trial of anethole dithiolethione in smokers with bronchial dysplasia. *J Natl Cancer Inst* 2002;94:1001–9.
- Veronesi G, Szabo E, Decensi A, Guerrieri-Gonzaga A, Bellomi M, Radice D, et al. Randomized phase II trial of inhaled budesonide versus placebo in high-risk individuals with CT screen-detected lung nodules. *Cancer Prev Res* 2011;4:34–42.
- Kim ES, Hong WK, Lee JJ, Mao L, Morice RC, et al. Biological activity of celecoxib in the bronchial epithelium of current and former smokers. *Cancer Prev Res* 2010;3:148–59.
- Mao JT, Roth MD, Fishbein MC, Aberle D, et al. Lung cancer chemoprevention with celecoxib in ex-smokers. *Cancer Prev Res*, In Press.

Conclusions

The iloprost trial is the first randomized, placebo-controlled trial to document a statistically significant effect of a chemopreventive intervention in improving bronchial histology, picking up an important new clinical signal. The full implications of this finding are evolving as we seek to understand the magnitude of the effect and its potential for translation to cancer prevention. In the meantime, ongoing analyses of the multiple specimens derived from this trial will help in defining the mechanisms of action of iloprost and in identifying risk and predictive markers and will add to the body of evidence surrounding the use of iloprost for lung cancer chemoprevention. The promising results of this well-done clinical trial, coupled with a convincing mechanistic rationale and consistent animal studies, identify iloprost as a promising chemopreventive agent and support further studies of iloprost and/or other agents acting via similar mechanisms in preventing lung cancer in former smokers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were reported.

Received April 13, 2011; revised April 23, 2011; accepted April 26, 2011; published online June 2, 2011.