Perspective

Assessing Efficacy in Early-Phase Cancer Prevention
Clinical Trials: The Case of Ki-67 in the Lung

Perspective on Kim et al., p. 148

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Abstract

This perspective on Kim et al. (beginning on p. 148 in this issue of the journal) examines the value of the Ki-67 proliferation index as a surrogate end point in early-phase clinical lung cancer prevention trials. The clinical trial of Kim et al. shows an effect of the cyclooxygenase-2–selective inhibitor celecoxib at a high dose on Ki-67 expression in the normal bronchial epithelia of current and former smokers. The critical issue of how these data can be used to further drug development is discussed. Cancer Prev Res; 3(2); 128–31.

Introduction

The transition from early-phase to definitive (phase III) clinical trials remains one of the most challenging aspects in the development of cancer prevention agents. The decision to proceed to a phase III trial, with its requirements of substantial resources and a lengthy time frame for completion, demands careful consideration of potential efficacy and toxicity. Assessment of efficacy is informed by data from a variety of studies, including in vitro mechanistic studies, testing in animal models, epidemiologic cohort and case-control studies, and early-phase clinical trials or secondary end-point analyses of well-designed trials done for other indications (1). By virtue of testing investigational agents in humans, early-phase clinical trials should provide the most informative data for deciding to proceed to the next step. The lack of validated end-point surrogates for cancer, however, has hampered the interpretation of early-phase (phase II) testing and thus progress in the field. In contrast to phase II cancer treatment trials, which use tumor measurements to assess agent efficacy, phase II cancer prevention trials do not have easily measured primary trial end points established for indicating preventive efficacy. Therefore, early-phase cancer prevention trials generally assess surrogate efficacy measures that are even more distantly related to the definitive end point of cancer incidence than tumor shrinkage is related to survival.

A number of cancer prevention clinical trials have used cell proliferation, as determined by immunohistochemical staining for Ki-67, as a potential surrogate end point. After more than a decade of accumulating data on the Ki-67 proliferation marker as an end point of early-phase lung cancer prevention trials, it is time to reassess how well this end point meets the general requirements for a surrogate end point (reviewed in refs. 2–4) and its utility for deciding if potential agents should proceed to definitive testing. Although this perspective will focus primarily on lung cancer trials, the same issues pertain to early-phase studies targeting other organs.

Ki-67 and Efficacy in Lung Cancer Prevention Trials

In this issue of the journal, Kim et al. report their examination of the effect of the cyclooxygenase-2 inhibitor celecoxib on the Ki-67 proliferation index in the bronchial epithelium (5). The authors found that a high dose of celecoxib (400 mg twice daily, the U.S. Food and Drug Administration–approved dose for colorectal polyp reduction in patients with familial adenomatous polyposis) statistically significantly reduced Ki-67 labeling in the bronchial epithelia of current and former smokers after 3 months of treatment. In contrast, a lower dose (200 mg twice daily) and placebo had no effect on Ki-67. Therefore, a biological effect on the bronchial epithelium was clearly shown with high-dose celecoxib treatment. The question that follows, however, is, "How do these results inform the decision to proceed with a definitive efficacy phase III trial?"

To be useful, Ki-67 or any other marker needs to meet several requirements (2–4). It should be integrally involved in the process of carcinogenesis, such that its expression correlates with the disease course. Its expression should differ between normal and at-risk epithelia, and it should be easily and reproducibly measurable in specimens likely to be obtained in clinical trials. Last, the expression of the marker should be modulated by effective interventions, and there should be minimal spontaneous fluctuations and no modulation by ineffective agents. A marker that satisfies these criteria then needs to be validated in prospective clinical trials (2). Because different classes of agents can act through different pathways, a marker...
that accurately predicts cancer incidence reduction with one class of agents unfortunately may not be as predictive, or not predictive at all, with a different agent class.

The value of the information obtained from Ki-67 as a modulatable end point in lung cancer chemoprevention trials has been reviewed previously (6, 7), and Ki-67 satisfies some (but not all) of the surrogate end point requirements outlined in the previous paragraph. Dysregulated proliferation is a well-established hallmark of carcinogenesis (8), is associated with a poor prognosis in lung cancer (9), and is increased in premalignant lesions in the bronchial epithelium (10, 11). The interpretation of the Ki-67 labeling index is complicated, however, by the ability of smoking cessation alone to decrease it, as shown by the different baseline expression levels in current versus former smokers in the studies of Kim et al. and others (5, 10). The time course of this decrease is not well understood, although measurements of Ki-67 and epithelial remodeling in patients with chronic obstructive pulmonary disease suggest that hyperproliferation resolves over a period of years after the patient stops smoking (12). The average number of cells expressing Ki-67 per millimeter of basement membrane among these chronic obstructive pulmonary disease patients was 18.6 for current smokers, 6.9 for former smokers who quit within 3.5 years, and 2.8 for former smokers who had quit for more than 3.5 years. Therefore, time since smoking cessation complicates serial Ki-67 measurements within clinical trials, and there seems to be considerable variability in the rate of decline in Ki-67 expression between individuals who have quit smoking (10, 12).

It is tempting to hypothesize that individuals with increased proliferation in their bronchial mucosa are at an increased risk of lung cancer (thereby supporting the converse hypothesis that Ki-67 reduction will be mirrored by a decreased risk of lung cancer), but supporting data are lacking. First, animal studies have shown that a number of irritants not necessarily associated with carcinogenesis, such as high oxygen content, ozone, sulfur dioxide, mechanical irritation, and infection, all increase lung epithelial cell proliferation likely without increasing lung cancer risk (13). Second, it takes many years before the risk of lung cancer is reduced after smoking cessation, notwithstanding the early decrease in proliferation (albeit not necessarily to “normal” levels) following smoking cessation (14, 15). Extended follow-up from the Lung Health Study showed that a decrease in lung cancer incidence was not demonstrable until 14.5 years after smoking cessation, well past the time when average Ki-67 expression has already significantly decreased according to other studies (16). Therefore, decreased Ki-67 was not mirrored by an early decrease in lung cancer risk. Miller et al. also found that Ki-67 expression in nonmalignant bronchial cells is not related to lung cancer risk, although these authors did confirm the risk association with histology and smoking (16). Of interest, these authors found a similar expression of Ki-67 in nonsmokers and former smokers and did not find any evidence of a continued decrease in Ki-67 expression with increasing duration of smoking cessation, suggesting that the main decrease in Ki-67 occurs in a relatively short time frame after quitting.

Taken together, these data indicate that Ki-67 is a dynamic index under the influence of a variety of external stimuli. Kim et al. have shown that high-dose celecoxib is one of these influences. Mao et al. (17) reported a similar significant decrease (35%) in Ki-67 in a single-arm pilot study of celecoxib in 20 current heavy smokers. These celecoxib studies show statistically significantly reduced Ki-67 across a relatively large population, with the larger Kim et al. randomized controlled trial confirming and expanding the results of the smaller nonrandomized Mao et al. study. However, variability due to the influence of a myriad of factors, such as infection and environmental exposures, indicates that Ki-67 expression is too subject to modulation by influences that do not affect lung carcinogenesis to be an accurate reflection of the modulation of lung cancer risk, at the level of either a broad population or an individual.

Understanding the Significance of Biomarker Modulation

A full understanding of the implications of Ki-67 modulation would require anchoring these data to either cancer incidence or at least another biomarker integrally linked to cancer incidence. The histologic end point of intraepithelial neoplasia (IEN) typically has been used as a surrogate for cancer incidence in phase II cancer prevention trials (18), although no data establish that regression of the IEN bronchial dysplasia can prevent invasive lung cancer. Studies of typical phase II end points such as IEN and Ki-67 nested within phase III cancer end-point trials are critically needed. Such an approach is being tested in an ongoing phase III trial that will simultaneously assess the influence of a preventive agent on the precursor IEN oral leukoplakia and on oral cancer incidence (19). This approach is much more difficult, however, when testing prevention of internal malignancies such as lung cancer, in which invasive procedures are needed to determine the status of the IEN. Validating surrogate end points in these settings most likely will require phase II biomarker studies involving agents with previously established effectiveness from phase III testing.

In the case of the celecoxib trial by Kim and colleagues, Ki-67 expression in nonmalignant epithelium is not linked to cancer incidence (because the trial was too short for cancer development) or to histologic premalignancy (because too few participants exhibited histologic abnormalities). Therefore, it is difficult to place these Ki-67 data within a larger context. This research team also assessed Ki-67 in a previous chemoprevention trial of retinoids, but this study showed significantly reduced Ki-67 staining only in the parabasal layer rather than throughout the thickness of the epithelium (20). Although different agents indeed may target Ki-67 in different epithelial compartments, these data suggest even less utility for Ki-67 as a broad marker for assessing efficacy across many agent classes. A truly useful surrogate marker should have similar
performance characteristics across multiple agent classes, or it will need to be revalidated each time a new agent class enters development. For this reason, class-specific markers are of limited use at this time for providing sufficient data to decide whether or not to proceed to phase III studies, which is the main utility of a surrogate marker.

Another major issue of concern is quantification—how much biomarker modulation is needed to determine effectiveness? This question has no easy answer. A statistically significant modulation gives a measure of comfort, but it is hard to understand what it truly means and how it would translate to predicting a reduction in cancer incidence. One could apply Response Evaluation Criteria in Solid Tumors-type criteria such as a 30% reduction in the biomarker value, but there are no functional data to support such an approach. In the case of a biomarker such as the Ki-67 proliferation index, one could seek a reversion to the expression level seen in truly “normal” epithelium. This would require having a comparison database of expression values derived from age- and sex-matched individuals who have no lung pathology whatsoever. It is very difficult to obtain bronchial biopsies from at-risk individuals, and the challenge in obtaining biopsies from normal volunteers is manyfold greater.

An alternative to single markers such as Ki-67 is a panel of markers or a high-throughput readout such as a gene expression or proteomic profile. Gene expression profiles associated with celecoxib treatment have been examined in tumor tissues in two preoperative studies (21, 22) and in histologically normal epithelium in another study (23). Auman et al. (21) primarily found changes in genes involved in cellular lipid and glutathione metabolism and in cell adhesion in colorectal adenocarcinoma tissue after 7 days of preoperative celecoxib. Sooriakumaran et al. (22) found modulation of genes mainly associated with apoptosis and tumor suppressor function in prostate cancer tissue after 28 days of preoperative celecoxib. Whereas both of these studies addressed gene expression in tumor tissue, Glebov et al. examined the effect of celecoxib (for 12 months) on gene expression in the normal colonic mucosa of patients with hereditary nonpolyposis colon cancer (23). These investigators found alterations in the expression of more than 1,400 genes, although the magnitude of change was less than 2-fold in the majority of genes. The changes mainly involved immune response, cell signaling and adhesion, response to stress, transforming growth factor-β signaling, and regulation of apoptosis.

These three studies involving different tissue compartments and durations of celecoxib treatment all found some effect on apoptosis and a potential effect on proliferation. Although an antiproliferative effect was hypothesized due to the identification of genes associated with decreased proliferation, none of these studies identified Ki-67 specifically. Of course, gene and protein expression do not necessarily correlate exactly. It is also conceivable that effects on gene expression differ in normal versus preinvasive versus invasive tissues in different target organs, making it difficult to extrapolate data from the colon and prostate to the histologically normal bronchial epithelium. Taken together, these three studies show that celecoxib treatment profoundly affects the target epithelium across at least two different organ sites, but they are unable to link the changes in gene expression to more definitive end points such as future cancer incidence and thus fall short, as does the Kim et al. lung/celexib study, of providing a sufficient justification for moving to phase III cancer prevention drug development.

Conclusions

What have we learned from studies using the Ki-67 end point, and how do these results inform the decision to proceed with a definitive efficacy phase III trial? As with the pilot human studies showing an effect of interventions on gene expression profiles (21–23), trials using the Ki-67 end point show a biological effect in the target organ. Demonstrating an effect in the target epithelium is a necessary early step in the development of cancer prevention drugs—it is highly unlikely that a drug could be effective if it does not reach its epithelial target. Inability to affect the target should be grounds for discontinuation of drug development. The Kim et al. celecoxib trial showed that low-dose celecoxib had no effect on Ki-67, suggesting that the low dose should not be studied further for this indication. Although high-dose celecoxib did modulate Ki-67 in the same trial, this type of information is insufficient for proceeding directly to a definitive phase III trial because it does not provide a full enough understanding of the corresponding biology. Inhibition of proliferation could theoretically be accompanied by an even greater inhibition of apoptosis, resulting in a net increase in abnormal cell number. Instead, demonstration of modulation of Ki-67 provides justification for additional phase II studies with biomarker end points that more closely approximate the definitive end point of cancer incidence. Similar to drug development for cancer treatment, it is likely that multiple early-phase trials will be needed before a go or no-go decision for phase III cancer prevention trials. With new high-throughput technologies such as gene expression arrays, a pharmacodynamic effect should be demonstrable with fewer study subjects and a shorter duration than typically required of phase IIb biomarker studies such as that by Kim et al.

Ki-67 should remain a part of the panel of markers to be evaluated in phase II trials, but it is insufficient alone to justify definitive phase III trials. The search for meaningful surrogates for cancer incidence therefore needs to continue and to accelerate. In the meantime, we still need to accumulate a full array of consistent information from epidemiologic, preclinical in vitro, and animal studies and probably several early-phase clinical trials before deciding whether a phase III clinical trial is justified or not.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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