Despite the successful demonstration of cancer preventive efficacy exemplified by agents such as tamoxifen and raloxifene for breast cancer, development of chemopreventive agents remains a difficult and challenging endeavor (1,2). Among the more daunting of the challenges is the identification of effective agents in early-phase clinical trials, before the sizable investment of resources required for phase III studies has occurred. The demonstration of preliminary efficacy requires not only an effective agent that is delivered appropriately for long enough to be effective but also the use of informative endpoints that predict the true endpoint of decreased cancer incidence. Whereas cancer treatment trials use surrogates such as tumor shrinkage to identify promising agents in phase II trials, cancer prevention trials seek to forestall the occurrence of future events (e.g., cancer). The absence of relatively easily measurable attributes of cancer development, such as tumor size, means that the surrogate endpoints used in phase II cancer prevention trials are, by definition, more distantly related to the true endpoint of cancer incidence. Identification and validation of such surrogates has been a major focus of chemoprevention research in recent years and remains one of the biggest roadblocks to chemopreventive agent development.

The study by Hittelman et al. (3) in this issue of the Journal examines the expression of the proliferative marker Ki-67 in the bronchial epithelium of former smokers taking 9-cis-retinoic acid (9-cis-RA), 13-cis-retinoic acid (13-cis-RA) plus α-tocopherol, or...
placebo to determine its utility as an endpoint in chemoprevention trials. The investigators show, in a per-site analysis, that both interventions statistically significantly reduced Ki-67 staining in the parabasal layer of the bronchial epithelium, including in histologically normal tissue. The implication is that successful modulation of Ki-67 points to a potential role for these retinoids in chemoprevention in former smokers. Although multiple aspects of this study deserve comment, the following commentary will focus on one main issue—what can we learn from modulation of a proliferative marker such as Ki-67?

The appropriateness of Ki-67 as a modulable biomarker in chemoprevention trials has been reviewed previously in the Journal (4), and not much has changed since that review. The Ki-67 protein is present in the nuclei of cells in all active phases of the cell division cycle, but it is not found in the G0 resting phase or during DNA repair (5). Ki-67 is expressed in cells in early G1 phase, before the restriction point or commitment to mitosis, as well as in cells that are arrested in G1 or M phase that have not finished transiting through the cell cycle. Thus, the Ki-67 labeling index is not a measure of proliferative rate or successful cell division but is rather a measure of the proliferating and potentially proliferating cell burden. This marker needs to be used cautiously to assess the effectiveness of agents that arrest cells at points in the cell cycle other than G0 phase.

Dysregulated proliferation is a well-established hallmark of carcinogenesis (6). Increased Ki-67 has been associated with worse prognosis in some studies of non–small-cell lung cancer [for summary, see Vourlekis and Szabo (7)], but it is not clear that Ki-67 is a marker of increased risk for lung cancer. Previous studies have shown that Ki-67 expression is increased in bronchial atypical lesions, such as metaplasias, compared with histologically normal epithelium in the same individuals, and expression is higher in current smokers than in long-term former smokers (8). Both smoking status and presence of preinvasive lesions are well-recognized risk factors for lung cancer (9,10). Ki-67, however, decreases within the first 6–12 months after smoking cessation, in association with the reversal of squamous metaplasia (8)—this time frame is faster than any appreciable decrease in lung cancer risk and raises questions about the independent prognostic value of Ki-67. Because some former smokers continue to exhibit elevated Ki-67 expression even after quitting tobacco use, long-term follow-up should be able to define whether the elevated Ki-67 expression is, indeed, predictive of future lung cancer risk.

It is of interest that both interventions in the per-site analysis reported by Hittelman et al. (3) were effective in reducing proliferation, which was a secondary endpoint in the study. The primary endpoint of this clinical trial, reported previously (11), was reversal of loss of retinoic acid receptor-β (RAR-β). This endpoint was reached only in the 9-cis-RA arm, not in the 13-cis-RA plus α-tocopherol arm. Because RAR-β was absent at baseline in only 30% of all biopsy specimens, the primary endpoint could be assessed only in the 63% of study participants who had at least one biopsy specimen that showed RAR-β loss. Nevertheless, if one assumes that modulation of either marker constitutes evidence for further drug development, then RAR-β and Ki-67 provide contradictory data about 13-cis-RA plus α-tocopherol. It is currently not clear if either marker is sufficiently informative to recommend further drug development decisions. This points to the difficulty of using biomarkers as primary and secondary endpoints in clinical trials when the underlying biology is incompletely understood, as is true for most epithelial cancers.

The value of surrogates needs to be tested in studies that assess both the surrogate and the true endpoint (12). In fact, both 13-cis-RA and α-tocopherol have been investigated for lung cancer prevention, albeit with different doses and not as a combination (13,14). Both studies were negative with regard to cancer incidence, the true endpoint. Unfortunately, the surrogate proliferative marker, which is assessed in bronchial tissue, cannot be integrated easily into a cancer incidence study in which bronchoscopic biopsy specimens are not routinely obtained. We therefore do not know whether Ki-67 was modulated in these studies by either drug alone or how it performs in comparison with the true endpoint of cancer incidence. The rationale for combining 13-cis-RA and α-tocopherol in the current study is based on better tolerance of the combination, allowing higher doses to be administered. Thus, it is conceivable that the combination could still be effective even though the individual agents are ineffective.

What have we learned? The chosen biomarker, Ki-67, is a modulable endpoint that was able to be assessed in all participants in the clinical trial. This achievement is no mean feat. Furthermore, both 9-cis-RA and 13-cis-RA plus α-tocopherol treatments produced a biologic effect in the bronchial epithelium, demonstrating that the drugs reached their intended site of action. Both of these components are necessary for effective drug development to proceed. Unfortunately, although they are necessary, they are not sufficient to justify the next steps in the clinical development of the agents that were tested. We do not have a full understanding of the effects of these agents on the bronchial epithelium or their effects during the full spectrum of carcinogenesis. As an example, it is conceivable that although these agents suppress proliferation in the histologically normal or mildly atypical metaplastic epithelium, they do not suppress proliferation in severely dysplastic epithelium that is more likely to progress to invasive cancer. Alternatively, the effect on proliferation may be offset by an even greater suppression of apoptosis, which could result in no effect or even an increase in cell number in the preinvasive lesions.

Surrogate endpoints are reasonable to use in phase II chemoprevention trials in which a false-negative result might limit the development of a potentially useful agent but a false-positive result will not result in widespread use of an ineffective agent because phase III definitive efficacy trials still need to be performed (12). However, in this era of rapidly increasing knowledge and limited resources for translational research, a large amount of internally consistent information from a variety of sources, ranging from mechanistic studies and preclinical animal models to epidemiologic data and early-phase clinical trials, is required to justify the transition to phase III drug development (15).

References


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