

Commentary

See related article by Armstrong et al., p. 410

Optimizing Biomarkers and Endpoints in Oral Cancer Chemoprevention Trials

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Abstract

Chemoprevention, defined as the use of natural, synthetic, or biologic compounds to halt, reverse, or prevent the initial phases of carcinogenesis or the progression of neoplastic cells to cancer, has produced successes, but progress has been slow. Notably, in the field of oral cancer prevention and despite extensive clinical investigations, a standard systemic therapy for patients with oral premalignant lesions is yet to be developed. In view of safety concerns surrounding the use of pharmaceuticals, the use of phytochemicals derived from the diet has been considered but has not yet translated into clinical success. The Bowman Birk Inhibitor (BBI) is a serine protease inhibitor isolated from soybeans possessing domains with trypsin and chymotrypsin inhibitory activity. Encouraging results were previously reported in a phase IIa trial of BBI complex in patients with oral leukoplakia with measurable clinical responses and favorable biomarker changes. In this issue of the journal, the less promising results of the randomized, placebo-controlled phase IIb trial are presented.

In this commentary, the complexities involved in defining optimal biomarkers and endpoints for oral cancer prevention trials and the development of dietary chemoprevention agents are discussed. *Cancer Prev Res*; 6(5); 375–8. ©2013 AACR.

Introduction

The use of intraepithelial neoplasias as a model to develop chemoprevention strategies, being that they represent near obligate precursors to and risk markers for invasive cancer, has been proposed and recently updated to include the molecular aberrations that are thought to represent hallmarks of the transformative process to cancer (1). Within this context, oral premalignancy serves as an ideal model for the study of cancer chemopreventive agents, as lesions are easily accessible for monitoring of clinical, histologic, and molecular responses to any given preventive intervention.

Several chemoprevention clinical trials for oral premalignant lesions have been conducted over the course of the last 4 decades and have investigated agents such as retinoids, cyclooxygenase-2 inhibitors, PPAR γ agonists, p53-tartgeted agents, EGF receptor (EGFR) inhibitors, and natural compounds (such as green tea extract and soybean-derived products). Unfortunately, so far, none of these studies have resulted in a pharmacologic strategy that could

be routinely used in clinic as a "standard of care" to effectively interrupt the process of carcinogenesis and development of invasive oral cancers (2).

In this issue of *Cancer Prevention Research*, Armstrong and colleagues report the results of another chemoprevention study in patients with histologically proven oral premalignant lesions (leukoplakia and/or erythroplakia; 3). In this phase IIb clinical trial, 513 people were screened over the course of approximately 10 years, and eventually, 132 were randomized to receive a Bowman Birk Inhibitor Concentrate (BBIC) versus placebo for at least 6 months. The primary end point of the study was clinical response. Secondary end points included histologic changes of the oral lesion by central pathology review and changes in buccal cell and serum HER-2 protein and buccal cell protease activity as pharmacodynamic markers. The clinical response end point was carefully defined upfront. Photographs of the oral cavity were obtained during the treatment course and blindly reviewed according to objective criteria as a secondary measurement of clinical response. Likewise, criteria for histologic changes were also defined in detail upfront, reviewed by a pathologist blind to the study arm, and scored according to a continuous millimetric scale of degree of tissue abnormality. Out of the 132 randomized patients, 89 were evaluable for the primary end point—28% of the subjects in the BBIC arm and 30% of the subjects in the placebo arm had a partial response or better, and the difference was not statistically different ($P > 0.81$). The assessments were confirmed by blind evaluation of the photographs. In both arms, there were no statistically significant changes in degree of tissue abnormality detected

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using the predefined scale. The authors concluded that there were no differences in the end points between the 2 groups, but the high response rates could indicate activity of the placebo used in the trial. In addition, 2 batches of BBIC were used over the long course of the study, and the second batch could conceivably have had reduced activity as a result of inadvertent storage under refrigerated and/or frozen conditions.

The study by Armstrong and colleagues has several strengths, including the use of carefully prespecified end points of clinical and histologic response with *post hoc* blind assessment by photographs and central pathology review, exclusion of noncompliant patients after a run-in period of placebo administration, and attempts at developing intermediate marker end points related to the mechanism of action of the study drug. Nonetheless, the study also illustrates challenges common to chemoprevention trials, including the complexity of developing natural compounds in this setting and selection of an optimal, clinically meaningful end point, as discussed below.

Natural Compounds for Chemoprevention

The suitability and selection of potential chemopreventive agents for clinical development, including those derived from dietary sources, may be determined by epidemiologic and preclinical studies. Natural compounds are attractive agents to be investigated in this setting, as they are usually perceived as safe (although this notion might be misleading; ref. 4) and are appealing to the general public, possibly facilitating accrual to clinical trials. Nonetheless, major barriers for development of natural compounds for chemoprevention include low potency, need for establishing standardized methods of production and storage to ensure constant concentrations of the active compounds in the formulation chosen (often with limited pharma support), reduced or unknown bioavailability, bioavailability affected by different genotypes of metabolizing genes, and most importantly, lack of understanding of molecular mechanisms of action and extensive preclinical testing showing efficacy in models specific to prevention.

Moiseeva and Manson have recently reviewed the characteristics of optimal preclinical development of natural compounds for chemoprevention (5). Of particular importance is attention to the use of meaningful doses of the chemopreventive agent to be studied. Often, doses higher than achieved with normal diet are explored to prove efficacy of the agent in preclinical models; these doses are usually not compatible with the epidemiologic data that supported the investigation of the natural compound as a chemopreventive agent in the first place. The dose choice is also complicated by the fact that a bimodal effect on cellular physiology may exist. Adequate time exposure in preclinical experiments is also key to allow for relevant translation of the findings to the clinic. Furthermore, culture conditions and choice of cell lines may significantly change conclusions of preclinical data—transformed cell lines are favored to infer effects of compounds on premalignant clones.

Finally, underpinning of the molecular mechanism of action may be challenging with the use of natural compounds. Several biologic effects may be observed in preclinical models, but the importance of each of these to halt carcinogenesis may be difficult to determine, thus hindering development of predictive biomarkers and intermediate surrogate markers of efficacy. It is suggested that high-throughput omic analysis preclinically may help elucidate the hierarchy of physiologic modulations that contribute to the chemopreventive property of the agent (6).

BBIC was tested for oral cancer chemoprevention based on epidemiologic data showing a strong relationship between ingestion of soybeans and low incidence of breast, colon, and prostate cancers. Soybeans have very high levels of protease inhibitors, and the protease inhibitor BBI is postulated to be the most potent anticarcinogenic agent in soybeans (7). Mucosa of smokers and patients with oral leukoplakia exhibit high levels of protease activity compared with nonsmokers (8). BBI reduced protease activity and incidence of invasive oral cancer in a 7,12-Dimethylbenz(a)anthracene (DMBA) hamster model of carcinogenesis (9, 10). A phase I study of patients with oral leukoplakia showed a single-dose troche of BBIC up to 800 chymotrypsin inhibitor units (CIU) to be absorbed rapidly and excreted in the urine between 3 and 12 hours after administration, without significant toxicities (11). Of note, average dietary intake of soy products in the high soy-containing Japanese diet is 200 CIU/day, indicating that BBIC supplementation in the clinical trials is not far from what can be achieved with diet alone. A follow-up phase IIa study showed clinical responses in 10 of 34 patients with leukoplakia treated with increasing doses of BBIC, with a dose-response effect (12). Changes in protease activity of oral mucosal cells after treatment correlated with modulation of cellular and serum HER-2 protein (13). The phase IIb study published in this issue was designed on the basis of these results and followed a stepwise, rational pathway of clinical development: initiating with assessment of epidemiologic/observational data, progressing to careful preclinical investigation, phase I/II testing with parallel biomarker validation, and ultimately, evaluation in a randomized study (3). Yet, results from these efforts failed to show that BBIC should be further developed and/or used for oral cancer prevention. Perhaps the greatest limitation of this and many other chemoprevention clinical trials is the selection of an adequate end point.

End point Selection for Oral Cancer Chemoprevention Trials

The natural history of oral premalignant lesions may vary according to several factors, including whether or not they are related to tobacco use, type of tobacco consumed, and continuation of exposure to the causative agent. In population-based studies in India, spontaneous regression of leukoplakia has been observed in approximately 30%–40% of patients (14, 15). Leukoplakias caused by chewing tobacco or smoking pipes also seem to regress more frequently

than lesions in cigarette smokers (16). Tobacco cessation for 3 months has been associated with clinical improvement of leukoplakia lesions in 56% of patients, and this figure rises to 80% with one-year tobacco abstinence (17). Similarly, complete or partial resolution of leukoplakias were seen in 33.8% and 25.3% of individuals, respectively, after elimination of the causative agent in a 520 patient study in Hungary (18). The risk of malignant transformation of leukoplakia is extremely variable. In a pooled analysis of the literature, the risk of developing invasive cancer in patients with oral premalignant lesions was estimated at 1.36% per year globally (19), with significant differences according to the geographic region of the world where the studies were done.

Shrinkage of oral premalignant lesions has often been selected as the primary end point of many chemoprevention studies. We would argue that this strategy is suboptimal, given the high spontaneous regression rates, the fact that a minority of premalignant lesions will transform into cancer, and the fact that only a marginal correlation between leukoplakia response to chemopreventive agents and oral cancer risk has been seen in the largest and longest term clinical trial using retinoids in patients with oral premalignant lesions (20). Furthermore, because of the high correlation between removal of the causative agent and regression of oral premalignant lesions, studies focusing on this end point should also carefully document tobacco and alcohol cessation and factor in this potential source of bias when interpreting the results.

Histologic response has also been used as a primary end point for oral cancer chemoprevention trials and was also evaluated as a marker of activity in the BBIC study reported by Armstrong and colleagues (3). Although histology has frequently been used to guide more aggressive surgical management of patients with oral premalignant lesions in the clinic, the relationship between degree of dysplasia and risk of invasive cancer has been seen in some, but not all studies (21–23). In addition, there is a high interobserver variability in grading of dysplasia (24–26). Furthermore, it has been documented that following chemoprevention intervention, molecular abnormalities associated with progression to cancer still persist in the histologically normal mucosa (27), suggesting that improvement in histology alone may not translate into a long-term reduced risk of oral cancer.

Taken together, the data discussed above lead us to believe that clinical or histologic responses of oral premalignant lesions to chemoprevention agents would only be clinically meaningful end points if they were surrogate markers of invasive cancer. Prentice and colleagues have discussed the properties of an ideal surrogate end point (28). In summary, the surrogate marker must be correlated with the true end point, must be modulated by the intervention, and must fully capture the net effect of the intervention on the true end point. To our knowledge, clinical and histologic responses of oral premalignant lesions have not been shown to entirely fulfill these criteria. Perhaps, in the future, modulation of molecular markers may serve as an early readout of oral cancer risk in patients with pre-

lignant lesions. However, in the absence of a valid surrogate, we suggest that invasive oral cancer be used as a main end point and reported in all later stage oral cancer chemoprevention trials.

Selection of invasive oral cancer as the main end point in this setting is, however, not without its challenges. In an unselected patient population, invasive cancer takes time to develop and is relatively infrequent, even in patients with an oral premalignant lesion. As a result, size and length of clinical trials using oral cancer as an end point will be extended and costly and will limit the number of agents that can be evaluated in this setting. Strategies to overcome these limitations include the selection of high-risk cohorts in which the frequency of the end point allows for an expedited reading of efficacy of the agent tested, using a relatively small sample size (29).

Recommendations

Molecular markers of cancer risk have been developed in patients with oral premalignant lesions and may serve as selection criteria for participation in chemoprevention clinical trials. Examples of such markers include chromosomal allelic imbalances, polysomy, p53, overexpression of podoplanin, p63 or EGFR, increased EGFR gene copy number, cyclin D1 polymorphisms, specific gene expression profiles, and specific DNA methylation profiles (2). Some of these have already been prospectively embedded in the design of newer-generation oral cancer chemoprevention trials (29).

We suggest that an ideal strategy to develop chemopreventive agents would include identification of a molecular marker that can be pharmacologically targeted by nontoxic drugs, actively participates in the carcinogenic process, is associated with a high cancer risk, can be modulated in preclinical experiments relevant to prevention where its modulation preclinically reverses one or more of the hallmarks of carcinogenesis, and preferably reduces incidence of invasive cancers in animal models. We envision that early clinical trials will focus on showing that the agent chosen to target the molecular marker is safe, can achieve cellular concentrations able to elicit biologic responses, and can modulate the target predicted to interfere with the natural history of premalignant clones. Phase 0 studies (30) could be a pathway to achieve these goals and could also identify possible predictive markers of response and surrogate markers of reduced cancer risk. In later-stage trials, the agent would be used in a larger number of patients in a randomized setting, using the definitive end point of invasive cancer, but also embedding secondary end points that could assist in validating the biomarkers discovered preclinically and during early-stage clinical testing.

Because of its complexity, this strategy may lead to a late-phase evaluation of only a few but the most promising agents. As illustrated by the study of Armstrong and colleagues (3), which accrued patients over the course of 10 years at multiple institutions, chemoprevention studies are far from trivial, and results can be disappointing even when the trials are designed based on solid evidence. As such,

optimization of resources and innovation using molecular-based approaches will be key to advance the chemoprevention field in the near future, especially as we enter a new era of genomic-based medicine.

Disclosure of Potential Conflicts of Interest

V.A. Papadimitrakopoulou is a consultant/advisory board member of GlaxoSmithKline, Amgen, Inc, and Genentech. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: W.N. William Jr, V.A. Papadimitrakopoulou
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.N. William Jr, V.A. Papadimitrakopoulou
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W.N. William Jr, V.A. Papadimitrakopoulou

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