

When "Effective" Prevention Agents Fail to Elicit Anticipated Effects: Challenges in Trial Design

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See related article by Jeter et al., p. 128

Introduction

In this issue of *Cancer Prevention Research*, Jeter and colleagues report a randomized phase IIB study comparing a combination of topical diclofenac and difluoromethylornithine (DFMO) to each single agent administered to sun-damaged skin in 136 patients who completed the study over three months (1). The goal was to test whether this combination was superior to either agent alone in reversing karyometric average nuclear abnormality (ANA), a novel set of quantitative measurements of nuclear morphology, which has been shown to predict the clinical aggressiveness of cutaneous squamous cell carcinoma (2). Contrary to expectations, the ANA increased in all three groups raising potentially important questions about the inherent efficacy of these agents, the status of neoplastic risk in the study cohort, the validity of ANA as an intermediate efficacy marker, and/or the impact of combinations of these variables on the ability of the study to answer its foundational question regarding the short-term efficacy of the combination relative to each agent alone. Although the results were interpreted as null, they could also be considered as an insufficient test of efficacy; in any case, they are certainly also informative regarding the many challenges of clinical chemoprevention trials. Here, we summarize some of these points to highlight the challenges and better inform future clinical research projects and designs.

Rationale

With the possible exception of NSAIDs, few compounds have elicited as much interest as DFMO in chemoprevention. As an irreversible inhibitor of ornithine decarboxylase, DFMO suppresses a critical target of multiple oncogenes as well as the rate-limiting step of polyamine synthesis (3). DFMO has elicited a significant amount of interest in colon cancer chemoprevention primarily because of a major trial showing a greater than 60% reduction in adenoma recurrence over 3 years and 92% reduction in the number of patients with advanced adenomas (3). In chemoprevention of skin cancer, systemic DFMO has shown an effect (though not statistically significant) in suppressing new basal cell carcinoma formation over

4 to 5 years (4). Upon follow-up, statistically insignificant effects of suppressing nonmelanoma skin cancers persisted without toxicity (5).

Topical DFMO reduced the emergence of new actinic keratosis by 23.5% when used over a period of 6 months and, importantly, was accompanied by a reduction in spermidine concentrations in the skin, although not in two other polyamines (6), as well as reductions in numbers of cells with aberrant p53 expression (7). Importantly, ANA karyometry has been used to measure effects of topical DFMO on moderately sun-damaged skin in combination with triamcinolone over 6 months, resulting in a significant reduction of karyometric abnormalities in all active (but not placebo) treatment groups (8).

Diclofenac is a pan-COX inhibitor that is FDA-approved for the treatment of actinic keratosis. Several randomized placebo-controlled clinical trials have demonstrated efficacy of the topical diclofenac formulation for this indication. However, comparing diclofenac with other agents such as 5-fluorouracil or imiquimod across their independent efficacy trials, it is likely that it is not as effective overall as other agents, although no direct, randomized comparison trials have been performed (9).

Biochemical and Surrogate Endpoints

Jeter and colleagues used an endpoint of quantitative nuclear karyometry to evaluate sun-damaged skin in this trial and the response to the interventions. This technology quantitatively evaluates nuclear morphology using 93 characteristics of nuclear chromatin patterns, including the ANA. Inflammation is a potential confounder of some of these measures; therefore, the authors focused on total optical density, relative nuclear area, and the number of densely stained pixels, which are presumed to be unaffected by inflammation. Even so, no significant differences between the arms were noted, which was certainly not anticipated. Given the fact that ANA has been established as an intermediate efficacy marker earlier with DFMO and other agents, what explains this finding? Importantly, the baseline ANA was quite low across the entire cohort in this study, suggesting that one of the issues may have been insufficient baseline actinic damage to observe an effect, if present.

In addition to nuclear karyometry, biochemical endpoints for the activity of DFMO were included in this study in an attempt to assure target modulation, which were polyamine assays for putrescine, spermidine, and spermine levels. Once again, surprisingly, these showed no significant differences across the treatment groups. There was no similar direct evaluation of the biochemical target engagement of diclofenac, such as COX-2 activity or tissue prostanoid concentrations, although Jeter and colleagues measured levels of COX-2 expression, which reflects tissue inflammation. Importantly, levels of COX-2 have been reported to decrease in human skin following diclofenac exposure (10), but it is not known whether this predicts clinical efficacy as measured by actinic keratosis regression.

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doi: 10.1158/1940-6207.CAPR-15-0432

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One of the important lessons learned from the pivotal adenoma trial using DFMO and sulindac was the dose deescalation approach designed to identify the lowest dose of DFMO that still yielded evidence of target biomarker modulation (3). Demonstrating that target engagement in skin occurred following DFMO and diclofenac administration would have been helpful in explaining null effects of the trial on ANA. However, the selected biochemical markers are inherently dynamic and given that they were measured in skin biopsies obtained one to two weeks following drug exposure here, leaves us unclear if the absence of ANA modulation derives from insufficient dosing of one or both agents, or whether the tissue sampling was simply too remote from the time of agent administration to demonstrate biochemical modulation.

Trial Design

This trial was not placebo-controlled, which did not allow for a baseline assessment of the natural history of sun-exposed skin over the 3-month period, a potential placebo effect notwithstanding. In addition, previous trials of topical DFMO were of 6-month duration. Therefore, it is possible that insufficient time elapsed to establish an effect, and the presence of a placebo control may have shown that the interventions produced a reduced rate of ANA aberrancy over time. Inclusion of a placebo arm to assure the natural history of neoplasia is often a valuable addition to such a trial, and it can typically be accommodated via unbalanced randomization (e.g., a patient distribution of 1:4, "placebo" to "active") if patients or an investigator are concerned about the inclusion of a placebo arm.

Multiple other unforeseen and potential problems may have also affected the ability to detect a potentially positive outcome. First, the vehicle for topical DFMO differed from that reported previously, and instead a commercial product, Vaniqa, was used due to concerns regarding the stability of DFMO in the investigational ointment. Without a direct comparison of biologic endpoints, it would be difficult to conclude definitively that the same amount of effective drug was delivered in this commercial formulation versus the investigational formulation of the prior study.

There have been reports of sex-specific differences in the efficacy of diclofenac in UV-exposed mice, in which reductions in tumor burden were observed exclusively in male mice following exposure to topical diclofenac (11). This brings to mind the issue of study populations. It is certainly possible that men may be more likely to benefit than women and as discussed above, that the baseline level of actinic damage, as quantified by karyometry, may need to be higher to see a therapeutic effect of DFMO. Other potential issues such as medication adherence could be an issue as well, especially as this was not a therapeutic trial of symptomatic patients benefiting from treatment, and rates of potentially irritating dermatologic side effects (i.e., dermatitis and pruritus) were substantial, especially for diclofenac.

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Safety

The investigators reported no significant safety concerns, but Fig. 1B shows that there was a substantial difference in those not completing the trial, 9.6% in each of the single-agent arms and 19.2% in the combination arm. The reasons for this difference are unclear, given that Table 4 suggests that dermatologic adverse events were of somewhat greater incidence and severity in those receiving diclofenac alone. Was the apparent difference due to the frequency or severity of nondermatologic adverse events, chance, or something else entirely?

Conclusions

Optimally, drugs used for cancer chemoprevention should meet requirements that are quite distinct from chemotherapeutics. In addition to proving efficacy in suppressing cancer development or progression, chemopreventive agents should be safe enough for long-term administration in individuals who may have no evidence of symptomatic disease. As cancers typically have lengthy latencies, these challenges are often evident, but potentially addressed through the use of intermediate efficacy biomarkers at the cellular, biochemical, and/or molecular levels.

The conclusions of this phase IIB trial suggest that further study is necessary to firmly establish the potential efficacy of combination diclofenac (or NSAIDs more generally) and DFMO in prevention of skin cancer and the relative efficacy of the combination versus that of the individual agents. A number of factors should be considered to determine whether a positive effect might have been masked in this instance, including the length of the study (i.e., 30 vs. 90 days), patient selection (i.e., patients with too little nuclear morphometric aberrancy at baseline), the validity of the intermediate endpoints assessed relative to the long-term goals of cancer prevention, and whether target engagement actually occurred (i.e., there was no biochemical modulation of polyamines or COX-2 expression, but was that due to insufficient dosing, the delayed timing of biopsies, or relatively insensitive makers in the case of COX-2 expression?) with the tested regimen.

Moving forward, it is possible that controlling inflammation either through dose reduction or concomitant use of steroids may be required to use karyometry as a robust measure of cancer risk, although steroids also have chemopreventive effects in some settings. A deeper mechanistic understanding of the relevant downstream molecular targets of DFMO may also be useful not only to identify potentially better biomarkers but also to identify better agents. Individuals at highest risk for developing skin cancers are most likely to benefit from chemopreventives in the short term, and it may be more straightforward to demonstrate positive findings in these individuals as well.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received December 14, 2015; accepted December 22, 2015; published online December 29, 2015.

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