Counterpoint: Because Some Surrogate End Point Biomarkers Measure the Neoplastic Process They Will Have High Utility in the Development of Cancer Chemopreventive Agents against Sporadic Cancers

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Introduction

The accompanying commentary (1) makes a valid observation that molecular or prehistological SEBs (2) thus far have had limited utility as end points for trials of chemopreventive agents against sporadic cancers. The commentary clearly identifies many of the hurdles facing the use of such markers for drug approval and the inherent need and challenges related to “validating” such markers. Importantly, the value of biomarkers in the context of mechanistic drug development is noted. Regarding the impact of histological biomarkers as legitimate end points for drug approval, the commentary tacitly acknowledges the current role of IEN as targets of medical intervention to reduce the risk of an individual for developing cancer. However, the authors are perhaps too pessimistic in their assessment of the use of IEN as the first generation of end points that ultimately will be refined by the use of molecular markers, similarly to the use of lipid biomarkers in treatment of CHD.

IEN have provided the basis for five drug approvals. These include celecoxib for regression of colorectal adenomas in FAP (3, 4), topical diclofenac and 5-fluorouracil in skin for treatment of superficial bladder cancers (5), and tamoxifen for prevention of invasive disease in patients with breast ductal carcinoma in situ (6, 7). This commentary will focus on the use of IEN and their refinement with markers of the disease process of carcinogenesis. It is also informative to point out that IEN and molecular and cellular biomarkers have already been very valuable in early and midstage drug development to help make go/no go decisions regarding continued development (8, 9).

Many of these markers besides IEN ultimately will have potential to be validated as SEBs, because they measure effects on fundamental properties of neoplasia (proliferation, apoptosis, and angiogenesis) and on specific drug effect markers associated with neoplasia (cyclooxygenase-2 activity and polyamine synthesis).

As also recognized by Armstrong et al. (1), the failures of biomarkers other than IEN to qualify as valid SEBs derive in part from the complexity of neoplasia as well as the need for SEBs to “predict patient benefit with reasonable certainty.” The failures result from the fact that the disease of cancer is tissue-based, and SEB development has been constrained by naive approaches to modeling the disease and its multipath, multifocal development process with isolated molecular and cellular events. Furthermore, for biomarkers to be useful, the techniques for their determination need to be robust and exhaustively validated. When using biomarkers in studies, investigators need to adhere strictly to validated methods so that there is confidence that what is measured is consistent across studies. Achieving this objective may require extensive efforts such as those used to establish standards for the determination of cholesterol. The criteria for biomarker measurements have been the subject of many reviews (e.g., Refs. 8–14), yet much of the lack of progress derives from faulty adherence to these methodologies. Nonetheless, a sound scientific basis now exists to characterize SEBs for developing drugs (8). As described in the following paragraphs, there is every expectation, given the rapid pace at which new technologies and knowledge are evolving, that the characterization and application of biomarkers will become more efficient and exact, with more rigorous ways to use and validate new SEBs, thus providing insight into their clinical utility. Therefore, this commentary will briefly discuss the difficulties of measuring neoplasia, the certainty of IEN as validated SEBs, the issues of validation (and causality), the approach of using IEN and the genetic progression models to create and validate the SEBs of the future, and the critical and productive role that systems biology and new imaging and genomics/proteomics-based technologies will play in rapidly expanding the applicability of new SEBs.

Cancer as a Complex Stochastic Process

The potential multipath, multifactorial nature of carcinogenesis is foretold by the heterogeneity resulting from processing the human genome. The 30,000 or so human genes contain as many as several hundred thousand allelic variants from SNPs (this diversity is further expanded by splicing variants; Ref. 15). These variations are compounded another 3–5-fold by post-translational protein modifications leading to a multitude (>10^6) of protein-protein interactions (e.g., 16). Even if only a small fraction of the genome is critical to cancer, the number of
possible molecules and interactions involved is enormous. On a cellular functional level, this complexity is manifested by the intricate relationships among factors on signal transduction pathways such as described by Grandori et al. (17) for the Myc/Mad/Max network. Hanahan and Weinberg (18) elaborated on the complexity involving facilitation by activity in and cross-talk among four cell types (epithelial cells, fibroblasts, endothelial cells, and immune cells) within tissue microenvironments.

This level of complexity highlights the uncertainties of using isolated molecular and cellular biomarkers to measure carcinogenesis. Moreover, this complexity is heightened by expected intra-/intersubject and tissue variations. Nonetheless, the increasing understanding of genetic progression in cancer (e.g., Refs. 19, 20) and of signal transduction (18) in cancer target tissues and observations of genotypic changes characteristic of selected cancers (e.g., 21, 22), combined with the advances in technology for measuring and characterizing changes in gene and protein expression (16), suggest that analyses of such patterns of gene expression have the potential for development as SEBs. For example, the progress made in gene chip technology suggests that within a few years it will be trivial to measure 6–12 genes defining a genetic progression model (23). As for all of the SEBs, the feasibility of gene expression patterns as SEBs will depend on careful evaluation in the context of carcinogenesis.

IEN: The Integrative Power of Structure to Predict Function

No serious student of cell biology or pathology questions that the morphological changes associated with IEN are part of and predict the cancer process, and that the lesions of genetic progression manifest themselves as abnormal structure in the cytological abnormalities of neoplasia: increased nuclear size; abnormal nuclear shape; increased nuclear stain uptake; variations in cellular size, shape and stain uptake; increased mitosis; abnormal mitosis; and disordered maturation (differentiation; Ref. 24). IEN have been studied for decades and are known to be high-risk predictors for and near-obligate precursors to invasive cancer; they are now being recognized as diseases (9). One important criterion for evaluating a SEB is how well it encompasses the attributes of the clinical end point, in this case the cancer (reviewed in Refs. 8–14). Carcinogenesis is progressive and so are dysplasia (IEN). For most IEN, increasing severity of dysplasia is associated with increasing risk of progression to cancer. For example, it is far more likely that high-grade squamous intraepithelial lesions (CIN2/3) will progress to cancer than the low-grade lesion, and so is likely to be more easily validated as a SEB.

As acknowledged by Armstrong et al. (1), because there is an accepted and often known probability that their presence will lead to cancer, IEN are already accepted as validated end points for measurement of cancer risk reduction by both surgical and drug intervention.

Stochastic Processes Only Have a Probability of Reaching Their End Point. Therefore, Validation of Any Element of the Process Must Follow Probabilistic Rules

It is often observed that candidate SEBs are expressed at higher incidences than the cancers they approximate. Such will always be the case when there are completely unrelated end points (other causes of death) that do not allow all of the carcinogenesis to go to completion. On the basis of existing disease models, it is likely that all high-grade IEN carrying confirmed genetic lesions would end in cancer, if the host lived long enough (8, 19). Validation, like causality, is a relative term that only becomes absolute when all of the variables and elements of a process are known and can be studied quantitatively. It is undesirable and short-sighted to require any data more rigorous than validation based on probabilistic estimates, which is consistent with current medical and regulatory practice. On the basis of existing knowledge, we are assuming that even these early biomarkers are on one or more of the possible causal pathways to carcinogenesis. Because they are “far away” from the cancer end point, there is potential for interference, diversion, role in other biological processes, and so forth that would keep these early events from being ideal SEB. However, intervention to treat or prevent could be shown to provide clinical benefit, much as lipid-lowering has shown benefit in cardiovascular disease. If interventions are “safe” to the population at risk, it would even seem prudent to intervene.

Valued SEBs Will Proceed from Morphological Phenotype (IEN) to Morphological Phenotype-Molecular Phenotype to Molecular Phenotype as the Systems Biology for Cancer Development Is Explored and Understood

As validated SEBs, IENs are immediately useful in accelerating chemopreventive drug development. As we have stated above, the characterization and future development of molecular markers of carcinogenesis, and their future development and validation as SEBs will be most effectively done in situ within IEN. The additional development of genetic progression models will also proceed in this context, as it has from inception. There will be a point in the not too distant future, as the understanding of the minimum number of disrupted pathways yielding malignancy grows, that the patterns of change representing carcinogenesis will be relatively easy to measure. This process will evolve with the progress that is being made in understanding and analyzing systems biology. With this understanding will come SEBs in predysplastic tissue (a normal morphological phenotype), and the predictive value of these data will begin to exceed the predictive value of the abnormal morphology (IEN). This molecular pathology within IEN lesions, or even before appearance of these lesions, will also allow better identification of individuals at risk, improve study efficiency, and provide better quantitative estimation of drug efficacy than effects on IEN alone (8, 9).

IEN Are Better SEBs than Cholesterol

Because IEN is directly on the causal pathway and is closer in disease progression to cancer than is elevated cholesterol to cardiovascular disease, the arguments for IEN as SEBs for cancer are more compelling than those for cholesterol as a stand-in for CHD. Despite the large body of data showing the relationship of blood lipid-lowering to reduced risk of CHD, there are many individuals for whom the correlation is not seen. In other words, when the parameter evaluated is one of several early in the multifactorial disease process, other variables (for CHD, systemic blood pressure) along with secondary or indirect processes contributing to the disease (for CHD, examples are smoking history and diabetes mellitus) or protecting the subject may confound interpretation (25). Cholesterol does have some significant advantages over IEN. It can be measured frequently using a noninvasive method, responds relatively quickly to intervention, is easily quantified, and is measured...
using well-standardized and validated assays. In contrast, current methods of measuring IEN are difficult. Particularly, there are no reliable noninvasive techniques for evaluating IEN; it is extremely arduous to accurately measure IEN in tissues where the lesions cannot be easily visualized (breast, prostate, ovary, and pancreas). However, new technologies such as in situ image-based methods are available in many of the target organs (colon, bladder, bronchus, and skin), which could help remedy this situation (26, 27). In addition, waiting for IEN to regress or new lesions to develop can take months as opposed to the few weeks it takes to see changes in cholesterol levels.

In contrast to the position taken by Armstrong et al. (1), we believe that accumulating evidence indicates that new technologies for screening ultimately will make studies more efficient and lead to the development of more effective chemopreventive drugs. Particularly, improved technologies for targeting specific disease states that identifying subjects at high risk and/or those likely to respond to a specific drug have high promise. For example, Gleevac was approved on the basis of cytogenetic and hematological criteria for reduction of chronic myelogenous leukemia in patients who carried the characteristic bcr-abl lesion that the drug targets (28). Recent data suggest that bcr-abl transcripts are reduced in these patients (29, 30), although they may not be completely eradicated. In other words, molecular remission, i.e., reduction of bcr-abl transcripts, is a potential and very specific SEB for this drug. More directly related to chemoprevention and to SEB, celecoxib was approved for treatment of colorectal polyps (IEN) on the basis of a small, but carefully designed study in 81 subjects with the germ-line mutation for FAP. This relationship provides the rationale for evaluating celecoxib in the much larger population at risk for colorectal cancer, but who do not bear the germ-line mutation for FAP.

Noninvasive SEB Are Coming

Besides the great strides being made in tissue and molecular imaging, functional genomic and proteomic research holds promise for the development of noninvasive SEB. Of interest are the identification of markers in circulating DNA and cells (31) and the in situ imaging of gene expression (26). The work led by Petricoin, Kohn and Liotta (16, 32), in prostate and ovarian cancer, and that led by Staude (33) in leukemias and lymphomas, will mark these tissues as initial targets for development of genomic- and proteomic-based molecular profiles for identifying high-risk subjects and for monitoring effects of drug intervention in early stages of carcinogenesis. The ability to measure the functional effects of drug intervention will also be important to chemopreventive drug development. For example, a recent study by Belhocine et al. using annexin showed the potential for imaging apoptosis as a measure of therapeutic effects in cancer patients (34, 35); similar technology could be applied to evaluating interventions in precancers.

Conclusion

The science and utility of SEB in the development of cancer chemopreventive agents against sporadic cancers are solidly established. The issue of validation is a relative one, and IEN are validated for most target organs sufficiently for establishing that their prevention/removal provide clinical benefit. With rigorous attention to methodology, and to emerging scientific data and new technologies, there is every expectation that validated SEBs will be developed now in the context of IEN. This development will improve the efficiency of clinical development of chemopreventive agents, better identify those who are likely to benefit (or not to benefit), while also opening the door to even earlier identification of individuals at risk (e.g., those with predysplastic molecular lesions that occur before IEN). The rapid pace at which systems biology and new technologies are developing will make SEB science a very productive and exciting area, but also highlight the need for careful validation of such markers in the context of clinical trials. Contrary to the contentions of Armstrong et al. (1), the prospects for SEB making cancer chemoprevention studies more efficient and effective are bright. However, we agree with these investigators that hard work and exceptional dedication to sound, standardized methods will be required to assure that the effort in developing SEB is fruitful. The eventual acceptance of SEB may entail more than scientific rationale. Scientific and regulatory policy changes may also be required (e.g., 14).

References


