Foreword

Chemoprevention is intervention with chemical entities that delay, arrest or reverse processes that might lead to genetic instability, cell proliferation and the progression towards a malignant cell. Unlike tests of chemotherapeutic drugs, where efficacy is based on reduction of disease or decreased mortality, tests for effectiveness of chemopreventive agents incorporate reduced cancer incidence as an endpoint and involve healthy individuals. Since the development of cancer may require from several years to decades, chemoprevention trials generally incorporate large study populations and are characterized by long trial periods. These properties of chemoprevention trials have encouraged investigators in the field to explore strategies that can reduce both the length of trial time and cost. This has led to measures such as increased use of surrogate endpoints and study designs that incorporate cohorts with increased risk of developing neoplasia.

The principal goals of this publication are to:
- update and clarify molecular and biochemical mechanisms of colorectal and breast carcinogenesis and utilize this information to elicit ideas for new targets and surrogate endpoints for chemopreventive drug effect.
- explore the scientific issues related to the development and testing of chemoprevention approaches to breast cancer and colorectal cancer, particularly among selected persons at high risk of these diseases.
- promote collaboration among scientists from the United States, Israel, and Europe to develop novel approaches to intervene in subjects at high risk for the development of breast and colorectal cancer.

This special issue of Journal of Cellular Biochemistry is one of a series representing manuscripts describing presentations at conferences sponsored by the Chemoprevention Branch of the National Cancer Institute (NCI). This conference also was characterized by vigorous discussion among the participants, especially on the following topics:
- What methods are optimal in choice of interventions for clinical development and risk reduction trials?
- Should inherited genetic mutation cohorts be foci of chemopreventive interventions?
- How can subjects for cancer prevention research trials best be recruited and retained?
- How should surrogate endpoints be identified and tested?
- What is the status of use of new genetic and pathologic detection technology?
- How and when should multiagent chemopreventive combinations be studied?

I. What methods are optimal in choice of interventions for clinical development and risk reduction trials?

A brisk discussion among the workshop participants identified a serious deficit in translational animal models of carcinogenesis for common solid tumors. While standard chemical carcinogenesis models have provided important information regarding the potential efficacy of chemopreventive interventions in humans, few completed risk reduction trials or surrogate intermediate endpoints trials in humans have been completed to validate their usefulness. Transgenic models have similar deficiencies.

Dr. John Baron (Dartmouth Medical School, Hanover, NH) contrasted preclinical results from four different methods used to assess anticarcinogenesis activity and compared these to outcomes from risk reduction trials in humans. From his discussion, the following assessments evolved:

Risk organ	Epidemiology	Biobanking*	Animals	Biomarkers
Skin	Works	Works	Doesn't work	Works
Lung	Doesn't work	Doesn't work	Works	Doesn't work
Colon	Doesn't work	Doesn't work	Works	Works

*Testing of chemopreventive agent anticarcinogenesis effects in vitro.

These results further emphasized the need to develop, characterize, and utilize animal carcinogenesis models. Most discussants agreed that preliminary data in animal models of carcinogenesis should be required prior to clinical development. Many were disappointed with the lack of correlation of epidemiologic data and chemoprevention efficacy. Improved epidemiologic methods , more emphasis on molecular epidemiologic studies or combined epidemiology and biomarkers research may enhance the epidemiologic predictions in the future.

II. Should inherited genetic mutation cohorts be foci of chemopreventive interventions?

With the exception of those employing familial adenomatous polyposis cohorts, few chemopreventive interventions have been proceeded to Phase II or III trials. The discussants were unanimous in suggesting that chemoprevention trials should be initiated for genetically identified high-risk subject cohorts. Such groups of subjects might include hereditary non-polyposis colon cancers, BRCA1, BRCA2, familial adenomatous polypsosis, and MEN syndromes. Due to the relative paucity of such individuals and families, large-scale collaborations will be necessary to recruit sufficient numbers of subjects to such intervention trials. Extensive discussions regarding the difficulties encountered in designing intervention trials with tissue endpoints, ethical difficulties in such trial design, informed consent and counseling issues, and methods of data analysis followed. Agent selection for colon cancer preventive intervention trials (e.g. NSAIDs, Cox-2 inhibitors, DFMO, curcumin) should be relatively straightforward given the availability of agents as potential interventions for breast cancer prevention.

III. How can subjects for cancer prevention research trials best be recruited and retained?

This topic generated discussion characterized by considerable diversity. International perspectives from Great Britain-Europe, Italy, Israel, and the United States provided unique comparisons of difficulties in the development, review, recruitment, and retention of subjects for cancer prevention trials.

The impact of news media upon the activation, recruitment and retention of interventional cancer prevention trials was recognized as a decisive recruitment issue in Europe. Dr. Andrea Decensi (European Institute of Oncology, Milan, Italy) compared recruitment to breast cancer prevention trials prior to, during and after a magistrate's investigation of this trial and the attendant publicity associated with this investigation. He also reviewed the strengths and weaknesses of the local human use review process and how this process impacted upon this trial.

The issues of subject recruitment, the ratio of subjects screened to subjects on protocol was described for numerous trials. A discussion of the problems of recruitment suggested that high screened to on study ratios are common but also related to design and work expectation of Institutions and study subjects. Multiple reasons for these differences including culture, individual investigator approaches, resources, and logistics were described. Of note was the discussion of recruitment and retention on the recently completed tamoxifen breast cancer prevention trial by Dr. Leslie Ford (National Cancer Institute, Rockville, MD). Dr. Ford pointed out problems such as low payment, inadequate coverage of subject expenses, and high quality control standards. With respect to the strategies employed, Dr. Ford was disappointed with the responses to news media efforts in recruitment, but was favorably impressed by the positive contributions of individual community organization grass roots efforts (particularly those associated with local religious organizations).

IV. How should surrogate endpoints be identified and tested?

Considerable discussion was devoted to fundamental research on potential chemopreventive targets and surrogate endpoints. This issue generated extensive discussion regarding the selection

of surrogate endpoints, quantitative methodology, validation of quantitative methodology, and analysis of the data. Discussions led by Dr. Yosef Yarden (Weizmann Institute of Science, Rehovot, Israel) on the mechanisms of ErbB-2/HER family control of cellular signal transduction pathways were of particular interest. The direction of specific or nonspecific immunotherapeutics or chemopreventives at these targets may provide a particularly effective new paradigm for specific preventive and therapeutic approaches.

Additional discussions were directed at issues of assay reproducibility as applied to clinical trials. Whereas many basic research laboratories perform analytical procedures such as HPLC, immunoassays, Western blots, and RT-PCR, few such laboratories perform these procedures as assays in support of clinical chemoprevention trials or drug development trials. Criteria for laboratory assays include validation of between and within day variability and reproducibility. Quantitation of molecular endpoints and tissue endpoints is a major challenge. Dr. Laurence Freedman (Shiba Medical Center, Tel Hashomer, Israel) summarized the statistical criteria for validation of assay methodology in support of clinical trials.

V. What is the status of use of new genetic and pathologic detection technology?

Dr. Fred Fox (University of California, Los Angeles, CA) presented commentary on the future of genomics technologies in chemoprevention research. He reviewed the current technologies that are applicable for rapid gene screening and detection, assessed their relative future potential as research tools for translational research, and described prospects for future collaborative opportunities to exploit these new technologies.

Recent transcription profiling studies employing oligonucleotide and cDNA arrays or SAGE have provided dramatic demonstrations of differential transcription rates in normal vs. neoplastic tissue. However, all samples studied to date appear to be from late stage neoplasia, and there is no assurance that studies of tissue samples from chemoprevention trial cohorts will yield information that is of sufficient specificity and sensitivity to be of value. Minisequencing methodologies employing primer extension or the use of molecular beacons may be particularly fruitful in identifying chemoprevention study candidates bearing cancer predisposing genetic polymorphisms. However, these methods may be too expensive for use in screens for gene polymorphisms as surrogate markers in chemoprevention trials. This would involve an endpoint of no or few new polymorphisms as a measure of efficacy, and application of this strategy requires a precedent demonstration that a specific new polymorphism arises in a specific at risk cohort. New methods based on massively parallel cloning and high throughput capillary sequencing or sequencing by hybridization may provide cost effective approaches to establish such precedents.

This commentary led to an intensive discussion among the participants weighing opportunities presented by these new technologies, as well as the risks, potential benefits, and costs of their application. While all discussants agreed that the time is right to exploit new genomics technologies, most were uncertain about proceeding with such methods. The current technology appears to be most useful in rapid screening for known genetic mutations. Its use as a detection method for surrogate markers from human tissue or cell samples needs to be examined and investigated further. Moreover, the application of these technologies may be compromised by current limitations in the ability to process tissue for study. If large tissue samples are employed, the presence of a small tissue focus carrying a significant polymorphism may be obscured by the dominant normal gene population; if smaller samples are processed, a small area(s) bearing a significant polymorphism may be missed entirely.

Dr. Charles Boone described the application of computed image analysis as a tool to obtain quantitative data from pathologic samples. Dr. Boone advocated the use of such technology to examine cellular morphometry as a method of quantifying premalignant changes in human tissue samples. The widespread use of such methodology will depend upon the ability to validate quantitatively and to then diffuse the technology to sufficient numbers of sites to allow for access by research groups.

VI. How and when should multiagent chemopreventive combinations be studied?

Preliminary, unpublished data suggest evidence of chemopreventive agent synergism in chemical carcinogenesis models. Dr. Dean Brenner (University of Michigan, Ann Arbor, MI) presented a proposal to combine non-steroidal agents based upon mechanisms of inhibition of various arms of the arachidonic acid metabolic pathway. Other investigators felt such an approach was too didactic and suggested combining compounds with different anticarcinogenesis activities with tissue surrogate endpoints would be just as productive. Still other investigators expressed the opinion that current animal models were insufficient for predicting synergism of chemopreventives and recommended the development of new animal models prior to proceeding with the assessment of combinations of new chemopreventive agents. Overall, the group supported the development of combination chemo-prevention agents with careful preclinical in vivo data followed by systematic phase I and II human clinical trials. The agents most commonly mentioned for combination studies in the colon included NSAIDs with DFMO, oltipraz, and calcium.

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