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Executive Summary of Cancer Etiology Think Tank

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For many years, the focus in carcinogenesis has been on initiation, and particularly on the direct induction of DNA damage by chemical carcinogens. While this is an important aspect of carcinogenesis, further progress depends on the use of a more balanced approach, emphasizing that the carcinogenic process is continuous and dynamic. Chemicals (both exogenous and endogenous) can contribute to tumor formation at any part of the process, and do so by interacting with a broad range of molecules including proteins, lipids and RNA, as well as DNA. For example, studies investigating the pro-carcinogenic effects of reactive oxygen and nitrogen species (ROS) (RNS) on cancer initiation and progression identified a large number of cellular damage effects along the progression continuum, including evasion of apoptosis, insensitivity to antigrowth signals, self-sufficiency in growth signals, limitless replicative potential, sustained angiogenesis, and tissue invasion.

If a new, more comprehensive approach to carcinogenesis is to be maximally productive, a Systems Biology approach will be needed to deal with complexities head-on, focusing not on individual components but rather on networks that can be measured, modeled, and manipulated. It will be necessary to generate very large, highly accurate datasets describing the behavior of all components in the system. This will require highly sensitive, newly available technology that can identify a range of biomarkers measuring exposure. Benefits that will flow from these broadened investigations include:

The identification of biomarkers useful for assessing risk, for earlier diagnosis and for measuring therapeutic efficacy using newly developed technologies with extremely high sensitivity.

An understanding of how endogenous and exogenous chemical exposure impacts repair pathways, epigenetic changes, and protein and lipid function, and how they alter cancer susceptibility, which will lead to novel targets for prevention and therapy.

Development of better strategies for chemoprevention, exposure avoidance, and healthy lifestyles

The Think Tank identified knowledge gaps and resources needed to improve prevention strategies and identify at-risk populations that can be summarized into 5 broad areas:

Chemical processes and pathways: Expand studies to include a broader spectrum of the effects of endogenous/exogenous chemicals and their reactions in the carcinogenesis continuum. Use a systems approach to interacting signaling networks at the cellular and microenvironment levels.

Biomarkers: Design approaches for the development, validation and application of chemical biomarkers of exogenous and endogenous carcinogen exposure. Use damage products measurements (e.g., DNA, protein, and lipid changes; urinary and plasma metabolites) for early detection, risk assessment, and monitoring therapeutic efficacy.

Models: Develop models with sufficient dynamic range to study combined chemical exposures and enable modulation of endogenous chemical products via knockdown,

pharmacologic, chemopreventive, or dietary manipulation. A range models are needed, from microbes through vertebrates to three-dimensional organ culture systems.

Technology: Enable collaborative access to newly developed, high sensitivity, high resolution, expensive instrumentation for high-throughput data collections.

Recruitment, collaboration and resources: Train the biologists, chemists and modelers who must work collaboratively on chemical carcinogenesis in an interdisciplinary environment.

Introduction

The classic Berenblum paradigm of multistage carcinogenesis, conceptually dividing the process into discrete stages of initiation, promotion and progression, requires extension and modification to take into account current advances in cancer research. It is clear that carcinogenesis is a continuous, dynamic process. Human cancers are caused or modified by exogenous and endogenous chemicals, and the same chemical can have multiple effects along the initiation to progression continuum. To eliminate the burden of cancer, research priorities must reflect the continuity and enormous complexity of the carcinogenesis process. Although the identification of exogenous carcinogens is nearly complete, the identification of endogenous carcinogens is not. In many cases, exogenous exposure and endogenous processes predispose to cancer through the same ultimate effectors, such as ROS and RNS. Similarly, much is understood about metabolic activation and detoxification, about DNA adducts, and DNA repair, but knowledge of the biological consequences of DNA adducts is incomplete, and the effects of carcinogens on other molecules, such as proteins and lipids, are largely unknown. Critical to further advances, both animal and human studies highlight the need to consider the timing and duration of chemical exposure. To understand the contributions and interactions of all these factors, a systems biology approach is essential.

Animal models suitable for studying chemical carcinogenesis are very limited. While genetic loss-of-function mutations (largely knockout mice) have provided extremely valuable insights into genetic factors in tumor development, these ablative models often do not replicate the effects of chemicals. For example, modulation (vs. ablation) of DNA repair pathways results from exposure to endogenous and exogenous chemicals. As a result, while most cancers do not exhibit mutations in DNA repair pathways, damage continues to accumulate.

A theme that permeated the Think Tank and can be seen in much that follows is the importance of having biologists and chemists work in close collaboration to maximize progress in the field. Biologists are familiar with the complex changes in cell biology and physiology that occur during cancer development and progression, and have expertise with *in vivo* experimentation. Chemists understand the reactive potential of carcinogens, have the ability to synthesize proposed intermediates in carcinogen activation, and have access to technology that can identify carcinogens and their metabolites. Ensuring collaborations between these groups is complicated by differences in scientific approach and, often, their location in different schools within a university.

The Think Tank recommendations can be summarized under five topic areas: I. Chemical processes and pathways; II. Biomarkers; III. Models; IV. Technology; and V. Recruitment, Collaboration, and Resources. Recommendations in each area are summarized below, followed by discussion points from the presentations and the literature.

I. Chemical processes and pathways: The field's research focus must expand to include a broader spectrum of effects resulting from exposure to endogenous and exogenous chemicals throughout the carcinogenesis continuum. Such investigations should include the consequences of exposure on RNA, lipids, and proteins, and epigenetic modifications to DNA

and protein. To accomplish this, it will be important to investigate the consequences of infection and tumor promoter exposure on cancer development and to identify signaling pathways that are either dependent or independent of reactive oxygen/nitrogen species (ROS)/ (RNS) and other effectors of inflammation. Interdisciplinary teams will use a systems approach to determine how cells and signaling pathways interact within target cells and in their tissue microenvironment in response to carcinogen exposure. To learn how committed stem cells in target tissues respond to chemical exposures and how such exposures modulate their function may be a key to understanding their resistance to therapy and their persistence following treatment-induced remission.

A) Carcinogen effects on RNA, lipids, proteins and DNA: DNA damage and mutation occur along the continuum of carcinogenesis, not just during 'the initiation phase'. The field has focused on DNA, but carcinogens also damage proteins, RNA, and lipids. DNA, protein and lipid damage recovery pathways intersect, and responses to protein and lipid damage are just as important as DNA damage in mediating cell recovery from exposure. Although protein damage has been understudied, recent work identified protein adducts as biomarkers. For example, a mouse skin carcinogenesis study detected 95% of the labeled carcinogen bound in damaged protein and most of the remainder in RNA; the smallest amount of the label was associated with DNA. Even less is known about the consequences of carcinogens on lipid metabolism, or the pro- and anti-apoptotic effects of peroxidized lipids. The role of bioactive lipids in signal transduction needs attention and recent technologies have now enabled an investigation of these processes. Other factors that should be considered are:

***DNA Adducts*:** DNA adducts can lead to p53 gene mutations in specific tissues like bronchial epithelium. The p16 gene is only methylated among smokers. Neither the tissue specific responses to exposures nor their underlying mechanisms are understood and they require investigation.

Pathways: When epigenetics, protein pathways, and apoptotic pathways, are considered, it seems it is not specific genes, but pathways that are consistently altered in carcinogenesis. To identify pathways that influence cell death and mutation, genomic phenotyping for damage sensitivity could be useful. It is important to keep in mind that more than 1000 yeast proteins are involved in recovery from some carcinogenic agents. Because several interacting pathways are involved in recovery from a carcinogenic insult, it is important to identify synergism among these recovery pathways.

Timing of exposure: The timing of exposure over the life course can be critical with respect to an individual's risk of developing cancer. Further, huge threshold differences in metabolism and detoxification may relate to differential gene expression at specific developmental stages; these differences are not well understood.

B) Role of diet, hormones and carcinogenic processes: Effective prevention requires the identification of exogenous and endogenous carcinogens and their interactions. Among other environmental agents, dietary substances may be a key factor. Dietary effects operate in a background of genetic differences and circulating endogenous hormones, both or either of which can alter an individual's exposure risk.

C) The roles of inflammation, ROS and RNS and microbial flora: Inflammation is "the perfect storm" for carcinogenesis, causing DNA damage and activating production of growth-stimulatory cytokines. Anti-inflammatory compounds have chemopreventive effects in animal models and humans. Recently, attention has turned to the role of inflammation, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), throughout cancer initiation and progression. ROS and RNS cause a variety of different types of cellular damage; in addition to increased mutation rates, cellular damage results in evasion of apoptosis, insensitivity to anti-growth signals, self-sufficiency in growth signals, limitless

replicative potential, sustained angiogenesis, and tissue invasion—all hallmarks of tumorigenesis. Declining defense mechanisms during aging may increase sensitivity to inflammation. A number of human cancers result from the combination of infection and carcinogen interactions. Infection contributes to inflammation, but pathogenic processes specific to certain infectious agents also play important roles in carcinogenesis. The synergy of the hepatitis B virus (HBV) with aflatoxin is striking--the occurrence of liver cancer increases dramatically in infected people. The appropriate analysis of these complex interactions requires a systems approach.

D) The microenvironment in carcinogenesis: Although only one cell type may be capable of forming a given tumor, chemical exposure of the microenvironment is likely to have direct and/or indirect consequences. Exposure of animals to a carcinogen that directly affects one cell type may indirectly influence other cells in the tissue environment. In terms of prevention, inhibition of IKK in target cells is more effective than its inhibition in the surrounding inflammatory cells. An analysis of the different cell types' sensitivity may reveal chemical interactions in specific genetic pathways.

E) Tumor promotion: If it is possible to distinguish between factors affecting tumor initiation (genetic changes) and those that give the target cell its proliferative advantage, chemoprevention strategies might focus on inactivating the latter. Carcinogens not detected by the Ames test are likely to be tumor promoters, and are likely to involve ROS. Dioxin is an example of a substance that causes no DNA damage, but is a potent promoter in human skin and liver, acting through a single receptor that alters gene products influencing apoptosis, ROS, and cytokines. Better assays to detect tumor promoters should be developed. Age and gender also influence tumor promotion and are relevant to exposure assessment. For example, the ability to remove oxidative damage from the prostate may diminish with age and the commonly observed loss of COX2 activity in prostate cancer. Age and gender effects are clearly illustrated in diethylnitrosamine induction of hepatocarcinoma.

F) The analysis of complex carcinogenic mixtures: The assessment of complex mixtures of compounds, such as tobacco smoke, remains a particularly challenging area that requires considerable attention and resources. There is a scientific consensus that mixtures need to be investigated, but methods do not exist to address the complexities inherent in such studies at budget levels that will survive the NIH peer review process. Until now, most bioassays have used single, large dose exposures to a single chemical. Findings from the analysis of a single adduct species in a clean system cannot be extrapolated to real life exposures. People are much more likely to experience combinations of low dose exposures. A consensus is needed on scientifically acceptable methods to study mixtures in cells and animals that take into account differences in susceptibility due to developmental stage, genetic polymorphisms, and gender.

II. Biomarkers: Although biomarkers were not initially a part of the Carcinogenesis Think Tank agenda, chemists and carcinogenesis specialists are especially well equipped to identify them since biomarkers represent a spectrum of chemically complex substances such as chiral lipids, and endogenous protein and DNA adducts. Biomarkers can be used to monitor exposure and treatment, and as tools for early diagnosis. The chemists' focus can now be expanded to detecting protein, lipid and DNA damage. Epidemiologists need biomarkers of past exposure history. A key issue in animal studies is to identify methods to serially sample the same animal using urine or plasma rather than having to serially sacrifice animals. This would dramatically reduce the number of animals needed in carcinogenesis experiments.

A) Assessment of past exposures: Samples collected during epidemiology studies can be used to determine past exposure history of the subjects once carcinogen biomarkers are identified. Although hair is regularly used for chemical analysis, its use for protein assessment is questionable. Tools with sufficiently high sensitivity to study biomarkers

reflective of low exposure levels are needed.

B) Detection of present disease: Since tumors have different patterns of protein expression than normal tissues, it should be possible to identify altered protein patterns in blood or urine as cancer biomarkers via proteomics. Biomarker development and validation require both animal and human studies. Markers that track disease processes or predict cancer progression would be especially useful. The study of liver cancer in China illustrates that a successful biomarker study requires a high risk population so that sufficient numbers are available for statistically meaningful results. The study revealed a non-linear interaction of HBV and aflatoxin in determining liver cancer risk.

D) Prediction of future disease: *Fortune* magazine suggested that "The NCI should commit itself to finding biomarkers that are predictive of cancer development." The development of DNA, protein and/or lipid biomarkers that indicate cancer potential is a major goal. They could provide targets for chemoprevention strategies and guide patient counseling on lifestyle choices. Biomarker identification teams should also identify mutations so that adducts can be correlated with key mutations.

III. Models: Discussion focused on animal models that have the potential to provide a mechanistic understanding of carcinogenesis. It is important to determine the appropriate model to use, since a given model may be useful for a particular organ system, but not for all. Two models illustrate available insights:

Hepatocarcinogenesis: Rat liver hepatocarcinogenesis was induced by genotoxic hepatocarcinogens and the initiated preneoplastic cells were isolated. In wild-type animals treated with a single *in vivo* dose of the hepatocarcinogen diethylnitrosamine (DEN), superoxide production by the Kupffer cells increased and enhanced DNA damage and nitrotyrosine in liver proteins. Phox-/- knockout mice treated with DEN showed less DNA damage and almost no nitrotyrosine production. It thus appears that the cell injury, DNA damage, apoptosis/necrosis, and proliferation result principally from increased superoxide release by Kupffer cells. This model illustrates the importance of interactions between different cell types during the first steps of carcinogenesis. Infection, intestinal problems and ethanol can also stimulate Kupffer cells, and thus influence carcinogenesis.

Genetically Modified Animals: When induction of NF-B in mice is accompanied by deletion of IKKin intestinal epithelial cells, tumor incidence decreases . NF-B, a transcription factor that regulates the expression of anti-apoptotic genes, decreases the susceptibility of cells to apoptosis, and may have a role in tumor promotion. In this model, IKK and NF -B provide a molecular link between inflammation and cancer. However, in models of hepatocellular carcinoma, IKK disruption increases tumor number and size, as well as increasing apoptosis. In organs that can regenerate, increased apoptotic cell death pushes more cells into the proliferation cycle. Hence, a complex relationship exists between apoptosis and tumor formation, and promotion.

A) Animal models—strengths and weaknesses: Animal studies, particularly using rodents, have been the backbone of chemical carcinogenesis research. New methods of genetic manipulation in these species, and particularly in mice, offer promising new opportunities for research. The NCI Mouse Models Consortium is a useful resource to the carcinogenesis community. Some newer mouse mammary tumor models are metastatic, resembling the human situation. Mouse models may prove useful in identifying the carcinogens that cause breast, bone, and prostate cancer. Interestingly, a mouse model that develops lung cancers in response to cigarette smoke involves many of the same genes associated with human lung cancers. Do these genes play a role in initiation and/or progression? However, studies in rodents also have limitations. For example, tests have revealed that only 40% of chemical carcinogens harmful to animals are also harmful to

humans, and at lower doses, only half of these were toxic in humans. Most mouse tumors are rarely metastatic or invasive, and mice do not develop gastric cancer. Additionally, mice have higher glutathione transferase levels than do humans, which limits their usefulness for some types of research. Gene knock-out mice are widely used, but there is some concern that the complete absence of a gene is not always a good mimic of a drastically reduced level of expression of the gene, a situation more commonly seen in carcinogenesis. Aflatoxin studies illustrate another limitation using selected animal models. Fisher 344 rats metabolize aflatoxin much as people do, but cannot be infected with hepatitis B virus (HBV). Thus, there is no animal model that truly replicates the interaction of HBV and aflatoxin observed in human studies, but one should be developed. In looking for models beyond rats or mice, what are the options? Lower eukaryotes can be useful, particularly where the specific mechanisms involved are known. In DNA repair, the choices are yeast, mammals, or *C. elegans*; little is known about DNA repair in *Drosophila.* The growing appreciation of the importance of stem cells in carcinogenesis suggests that *in vitro* studies of embryonic and adult stem cell cultures may be very useful. Currently, methods to maintain and manipulate such cultures are limited, but rapid progress is anticipated.

B) Inflammation is a confounding factor in animal studies: Even brief inflammatory episodes during the course of a carcinogenesis study can affect the outcome. The flora in animal facilities differ, so the occurrence of tumors may be high in one facility and low in another. The existence of at least 20 types of *H. pylori*, makes it possible to miss their presence in supposedly *H. pylori*-free animals. If the inflammatory agents (pathogens) are removed, the ability to generate the phenotype (tumors) may be lost. Despite its importance, few animal models are available to study inflammation.

IV. Technology: Think Tank discussions of technology focused on four broad areas: A) new technologies, B) shortcomings of techniques, C) sensitivity and other challenges, and D) fiscal constraints.

A) New technologies: Mass spectrometry has been the most widely used analytical technique in chemical carcinogenesis, and spectacular strides have been made in this area in recent years. Real-time mass spectrometry with blood flow detection is a reality. Various types of mass spectrometry have improved to the point that they are approaching their limit of sensitivity. New types of mass spectrometers include the exquisitely sensitive Fourier transform ion cyclotron resonance (FTICR); MALDI-TOF/TOF, which can characterize proteomes, lipidomes, and DNA adducts; and the high resolution triple quadrupole, which can quantitate lipids, DNA adducts, proteins and protein adducts. Current work involves increasing their specificity still further.

Proteomics, lipidomics, and related genomic-level, high-throughput analyses are needed in carcinogenesis, as they are in many other areas of cancer research. Mass spectrometry is a very useful technique in these areas, but more technologies are needed. Further techniques of protein analysis utilize radioactive labels to detect and pinpoint changes in protein patterns after a challenge. Stable isotope proteomes can be used as standards to run in 2-D gels. The proteomics laboratory at the University of South Carolina is using chromatography columns on a chip, which are more sensitive than existing separation techniques. These chips should be generally available in two to three years.

The importance of non-invasive analytical procedures has been emphasized above, and intravital microscopy offers substantial promise in this area. Two-photon microscopy can penetrate tissue, at least to millimeter levels. It is possible with this technology to measure activities spectroscopically, an ability which could be applied to look for a ROS spectral signature.

B) Shortcomings of techniques: There are many areas in which technological improvements are still needed. Inferring sequential changes in animals from serial sacrifice

has serious pitfalls. The development of non-invasive techniques to track changes in a single organism would substantially improve the quality of such data. In the case of an inflammatory response, for example, an adequate imaging methodology to obtain real-time records of organ-by-organ changes would be invaluable. 2-D gel analysis and protease digest proteomics are not quantitative. Even using antibody array information to detect up-regulation of proteins may fail, if it is applied at the wrong time, and information on labile protein modifications is very easy to lose during analysis.

C) Sensitivity and other challenges: Analytical and instrumental sensitivity was a recurring topic of concern. More sensitive mutagenesis assays are needed--current assays cannot detect mutations less frequent than one part in 10^6. For detection of many endogenous substances (such as endogenous vinyl chloride at the DNA and gene level), more sensitive and artifact-free instrumentation is required. Assessing the carcinogenic potential of low–dose exposures is difficult, as is interpreting the "U"-shaped curves which may result. Ongoing challenges for technology include methods to separate modified from unmodified adducts; the tools to do a mass balance experiment, looking at many pathways in a system such as chlorination and bromination; the chemical means to detect changes in real time, perhaps using *in vivo* probes; improvements in the chemistry of detection so that not only end products of a reaction are detected; and the means to link carcinogens clearly to disease or disease subtypes.

D) Fiscal constraints: Some sensitive instruments are costly to obtain and maintain. Researchers are slow to invest in technology not available within a P01 or RO1 budget structure, until its suitability to their research challenges has been well-established. As a result, MRI or PET scans are rarely done in animal facilities due to their expense, although MRI resolution can show oxidative stress levels, changes which might have regressed by the time animals were euthanized.

V. Recruitment, Collaboration, and Resources: Although not among the designated discussion topics, personnel and resources were regularly mentioned as factors impacting the success of research programs and the future development of the field.

A) Recruitment: Students perceive the field of chemical carcinogenesis as doing the same thing for the past 30 years--testing chemicals and not asking mechanistic questions. Academic structures can be blocks to cross training, although some institutions have integrated chemistry with biology or instituted interdisciplinary programs that expose students to research in carcinogenesis. T32 grants to support young people entering the field and multi-disciplinary training grants funded by NCI have been effective in training and recruiting new people, but more effort is needed in this area. Think Tank participants agreed that a combination of improved marketing approaches and funding can attract promising graduate and post-doctoral students for future leadership in the field of chemical carcinogenesis.

B) Consortia: Consortium grants, including those within a single institution, were attractive to some Think Tank participants. Sharing biological specimens, tissue arrays, databases, and other resources benefits the research community and should be required. NCI can assist by coordinating efforts to acquire and divide up tissue (especially human samples), and provide material to investigators as needed. Collaboration between chemists and biologists is also productive, and is relevant to the recruiting concerns voiced above. In the context of the roadmap initiative, some participants had done an exercise regarding inter-and trans-disciplinary research and how to form a team. The essential factor seemed to be that everyone must address the same, specific question. Other Think Tank participants expressed considerable skepticism about the benefits of consortia. Some advised caution in forming large groups across the country, which can be inefficient in getting information and publications together. In one opinion, the worst thing NCI could do is to have a big urine bank or blood bank--researchers should be closely associated with the collection process in order to know the source and storage conditions.

C) The Grant System: **A** major impediment to consortium formation is the lack of convenient funding mechanisms. NCI's strict rules for funding a program projects were viewed as counterproductive. One solution is for NCI to develop the needed infrastructure by funding individuals to develop these resources. Some believe there is a lack of knowledgeable study sections members. People with seniority and collaborative experience aren't on these sections, and conflict of interest requirements preclude participation by the most informed and up-to-date people because of their previous associations or collaborations.

Specific Recommendations for the NCI:

The Think Tank identified knowledge gaps and needed resources to improve prevention strategies and identify at risk populations. Technologies that expand discovery opportunities are now available.

Chemical processes and pathways:

Expand research focus from DNA adducts and mutation analysis to include a broader spectrum damage resulting from exposure to carcinogens throughout the continuum of tumorigenesis.

Develop a systems approach to interacting signaling networks at the cellular, microenvironmental and macro-environmental levels.

Expand studies of reactive oxygen species and other mediators of inflammation to identify the variety of cellular and microenvironment damage they cause.

Enable studies of low levels of exposure, in which perhaps 1 cell in 1000 is altered. DOE support of studies to look at the effects of low levels of radiation that cause no change in cell cultures could serve as a model.

dentify carcinogens and other etiologic factors in breast, colon, and prostate cancer.

Biomarkers:

Provide resources for the development, validation and application of chemical biomarkers resulting from exogenous and endogenous carcinogen exposure and their damage products (e.g., DNA, protein, and lipid changes; urinary and plasma metabolites)

Identify biomarkers for early detection, risk assessment, and monitoring therapeutic efficacy; identify markers that track past exposure to carcinogens.

Develop instrumentation for high-throughput sample processing, to permit multiplexing analytical procedures for biomarkers.

Models:

Develop models with sufficient dynamic range to permit analysis of combined chemical exposures; enable modulation of endogenous chemical products via knockdown, pharmacologic, chemopreventive, or dietary manipulation.

Provide more realistic funding for studies using genetically modified mice. In many cases, investigators must produce 3-4 times the required number of animals to obtain the desired genotypes. When modifier genes are found, follow-up studies are often not done because they can take three years and 1000 cages of mice.

Make effort to standardize animal nutrition, health status and knowledge of endogenous bacterial flora. Encourage studies in which all investigators use animals with the same microbial flora.

Use models of cancer progression, as well as initiation and promotion, for studies of combined etiological agents including mixtures. Inflammatory processes must be considered when designing cancer biology models and in implementing human translational studies.

Develop a useful spectrum of biological systems, including single cell-microbes, invertebrates such as *C. elegans* and *Drosophila*, vertebrates including rodents, and three-dimensional organ culture systems, which can be used to look at cell/cell interactions and to study tissue interactions.

Pay more attention to timing of exposure and the influence of developmental stages on carcinogenesis.

Develop a controlled carcinogen-induced tumor model in which proteomics and genomics can be used to look for chemical/biological interactions.

Technology:

Using available, newly developed high sensitivity, high resolution instrumentation, develop high throughput technology in **cooperative arrangements**. Due to their expense, requirement for high level expertise, and the need for quality control, such instrumentation must be shared.

Establish and support instrumentation centers within major institutions to make costly technology widely available.

Develop non-invasive technology (e.g. imaging and serum sampling) to track changes over time, (avoiding serial sacrifice); develop methods to do mass balance experiments, improve the chemistry to detect changes in real time (in vivo probes). Develop technology to mark tumor stem cells and initiated cells for visualization and isolation.

Design approaches to make stronger *in vivo* correlations between adducts, biomarkers, mutations, initiated and pre-neoplastic cells and cancer—a process limited, at least in part, by our lack of high-sensitivity mutational assays to extend dose-response curves for biomarker-mutation correlations.

Recruitment, collaboration and resources:

Support fundamental training in chemistry.

Support database development (an area currently buried in the depths of grant proposals) and mathematical modeling predictions of how biological systems respond to carcinogen exposure.

Increase support for developmental projects. These are currently ignored by NIH.

Support cross-training to emphasize the special role of chemists at all levels, including training grants and PI visits to collaborating laboratories

Maintain NIH sponsored synthesis of high quality standards (labeled and unlabeled, small molecule and macromolecular).