

Is It Time to Advance the Chemoprevention of Environmental Carcinogenesis with Microdosing Trials?

Perspective on Jubert et al., p. 1015

Thomas W. Kensler and John D. Groopman

Abstract This perspective on Jubert et al. (beginning on page [1015] in this issue of the journal) discusses the use of microdosing with environmental carcinogens to accelerate the evaluation and optimization of chemopreventive interventions. The need for chemoprevention of environmental carcinogenesis is considered, as are the structure of microdosing, or phase 0, trials, technologies required to conduct microdose studies in this context, and ethical concerns. We also reflect on what microdosing studies have taught us to date.

What Is the Need?

People are continuously exposed to varying amounts of chemicals or manufacturing by-products that have been shown to be carcinogenic in animal models; nearly 100 such compounds have been designated as human carcinogens by the IARC.¹ Exposures to these exogenous agents occur through the environmental vectors of food, water, and air. In most cases, reactive, DNA-damaging intermediates are formed from these chemicals during their metabolism and conversion into excretable, water-soluble derivatives. Exposure to environmental carcinogens has been estimated to contribute to a majority of human cancers, especially through life-style factors related to tobacco use and diet. Notable examples are the tobacco-related carcinogens [e.g., nitrosamines and polycyclic aromatic hydrocarbons (PAH); ref. 1], heterocyclic amines produced from sustained, high-temperature cooking of meats (2), and the fungal food contaminants aflatoxins (3). Mechanistic investigations coupled with molecular epidemiology have established the causative links between exposures to environmental carcinogens and increased risks for cancers. In many instances, the pathway to reducing the cancer burden from these exposures is clear in principle—eliminate exposure. However, many exposures are largely unavoidable, such as exposures to the aflatoxins and other mycotoxins in food (especially in underdeveloped regions of the world), or require substantial behavioral changes that are exceedingly difficult to implement in individuals or populations. It is imperative to develop new approaches to prevention that

can help in reducing the continuing burden of cancers resulting from exposures to environmental carcinogens.

Elucidation of the molecular mechanisms of chemical carcinogenesis provides insight into targets for chemoprevention. Scores of animal studies have indicated that prevention of cancers induced by nitrosamines, PAHs, heterocyclic amines, aflatoxins, and other carcinogens is potentially achievable by a wide range of chemical classes of anticarcinogens (4). Two overarching prevention strategies involve either blocking the acquisition of genetic damage from these exposures or enhancing mechanisms for delaying (by impeding proliferation) or even eliminating (by enhancing apoptosis) the propagation of damaged cells. The majority of randomized clinical chemoprevention trials conducted to date have used the latter approach, sometimes with great success, as seen with several antiestrogens, 5 α -reductase inhibitors, and cyclooxygenase inhibitors (5). These trials enroll individuals who are healthy but at a relatively high risk for cancer often due to presumptive precursor lesions. However, a few completed clinical trials have attempted to reduce the burden of DNA damage imparted by environmental exposures to tobacco carcinogens (6, 7) and aflatoxins (Table 1; refs. 8–12). The end points for these trials were short-term modulations of biomarkers of carcinogen metabolism and DNA adduct formation. Modulation of these biomarkers is presumptive evidence for cancer risk reduction, a concept that has been validated in many animal models (13). Many strategies for modifying the bioactivation and/or detoxification of these environmental carcinogens have been developed (14).

As highlighted in this issue of the journal, however, Bailey and colleagues (Jubert et al.; ref. 15) have used a different approach for blocking the acquisition of genetic damage; they tested the hypothesis that the formation of molecular complexes between the dietary carcinogen aflatoxin and chlorophyllin, a stable derivative of the plant pigment chlorophyll,

Authors' Affiliation: Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland
Received 9/18/09; revised 10/9/09; accepted 10/26/09; published OnlineFirst 12/1/09.

Requests for reprints: Thomas W. Kensler, Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205. Phone: 410-955-1292; Fax: 410-9550119; E-mail: tkensler@jhsph.edu.

©2009 American Association for Cancer Research.
doi:10.1158/1940-6207.CAPR-09-0232

¹ <http://monographs.iarc.fr/ENG/Classification/crthallist.php>

Table 1. Summary of phase II placebo-controlled, randomized clinical intervention trials using aflatoxin biomarkers as intermediate endpoints

Agent	Dose and schedule	Size	Duration	Biomarker modulation	References
Oltipraz	Placebo qd; 125 mg qd; 500 mg qw	234	2 mo	2.6-fold increase in urinary excretion of AFB-NAC at 1 mo (125 mg); 51% decrease in AFM1 at 1 mo (500 mg) 6% decrease in AFB-AA at 2 mo (500 mg); no effects on urinary genotoxicity or 8-oxodG	(10, 41, 42, 44, 45)
Chlorophyllin	Placebo qd × 3; 100 mg qd × 3	180	4 mo	55% decrease in urinary excretion of AFB-N7-gua DNA adducts at 3 mo	(9)
Broccoli sprouts tea (glucoraphanin)	Placebo qd; 400 μmol qd	200	14 d	9% decrease in urinary excretion of AFB-N7-gua adducts at 10 d; 10% decrease in phenanthrene tetraols	(8)
Green tea polyphenols	Placebo qd; 500 mg qd; 1,000 mg qd	124	3 mo	17- and 14-fold increase in urinary excretion of AFB-NAC at 3 mo; 42% and 43% decrease in AFM1; 49% and 43% decrease in 8-oxodG with 500 and 1,000 mg, respectively	(11, 46)
NovaSil	Placebo qd; 1.5 g qd; 3.0 g qd	177	3 mo	Reduction in AFB-AA at both doses; 58% decrease in urinary AFM1 at 3 mo (3.0 g); no effect on 1-OHP	(12, 47)

Abbreviations: AFB-NAC, aflatoxin-mercaptopuric acid; AFM1, aflatoxin M1; AFB-AA, aflatoxin albumin adduct; AFB-N7-gua, aflatoxin N⁷-guanine; 8-oxodG, 8-oxo-2'-deoxyguanosine; 1-OHP, 1-hydroxypyrene.

would impede the bioavailability of this carcinogen in the gut. Extensively used as an over-the-counter drug and as a food colorant, chlorophyllin is a well-documented antimutagen in bacterial and mammalian mutagenesis assays and exhibits striking anticarcinogenic efficacy in trout, rat, and other model organisms exposed to environmental carcinogens (16). The Jubert et al. study goes further in that it compared the effects of chlorophyllin with those of chlorophyll on aflatoxin toxicokinetics. Lower blood and urine levels of aflatoxin following these interventions are presumptive reflections of diminished carcinogen absorption and likely reduced DNA damage in hepatocytes and other target cells. What makes this study unique among prevention trials is, rather than relying on ambient exposures to aflatoxins, as done in an earlier, phase II trial of chlorophyllin (9), it administered a microdose of radiolabeled aflatoxin to assess the pharmacodynamic actions of the interventions on the toxicokinetics of the carcinogen.

To account for the large numbers of mutations reported in human cancers, Loeb has argued that the acquisition or expression of a mutator phenotype is an essential, perhaps rate-limiting step of cancer development (17). Therefore, inhibiting the accumulation of mutations could delay carcinogenesis. Assuming a 20-year average progression to clinical cancer in adults, even a 2-fold reduction in the rate of mutation accumulation could provide a substantial cancer delay of up to two decades. In light of this view, the design and utilization of agents that reduce DNA damage could delay the onset of cancer. Therefore, intervention agents such as chlorophyllin or others listed in Table 1 are undoubtedly needed. But, how best to optimize the selection of candidate agents and their utilization in the correct cohorts? Is there a need and a role for microdosing experiments with human carcinogens in healthy volunteers? If so, are there constraints?

What Are “Microdosing,” or “Phase 0,” Studies?

The U.S. Food and Drug Administration introduced and published in 2006 the *Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies* to expedite the clinical evaluation of new therapeutic agents. As described in this document, phase 0 studies are first-in-human, proof-of-concept trials that integrate qualified pharmacokinetic and pharmacodynamic biomarkers and occur earlier than do phase I trials in the process of drug development. Phase 0 therapy studies offer no benefit to participants (typically advanced cancer patients), in contrast to traditional phase I trials, which can benefit individual patients in addition to evaluating drug pharmacokinetics, safety, and tolerability and establishing maximal safe doses for subsequent phase II trials. Like phase I trials, however, phase 0 trials are designed to inform the design and conduct of subsequent trials. Historically, phase II trials became the starting point for characterizing the pharmacodynamic actions of new agents in humans. Phase II trials can be randomized, double-blinded, and relatively large, long-term, and costly, whereas phase 0 studies are far less costly in being limited usually to one agent dose in a small number of patients (generally fewer than 10) without therapeutic intent. Phase 0 drug levels can be less than 1/100th of the dose that is postulated to induce a pharmacologic effect. The upper boundary for a microdose is 100 μg. The primary study end points for microdosing trials include evaluating related analogs for selecting the lead candidate, modulating molecular targets, and agent pharmacokinetics (18).

Environmental carcinogens are not drugs, which are defined as “articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease” [Federal Food, Drug, and Cosmetic Act, SEC. 201. (21 U.S.C. 321)]. Nevertheless, can the

concepts and approaches of phase 0 microdosing studies be effectively and safely applied to the development of chemopreventive agents? Certainly, a standard phase 0 therapy trial design is not likely to be necessary or useful for chemoprevention, given the high safety profiles desired for chemopreventive agents. It is important to establish evidence of pharmacodynamic action early in the process, which cannot be achieved with a microdose of many, if not most, chemopreventive natural agents, a limitation that may not apply, however, to certain targeted novel preventive agents. However, can we reverse the usual phase 0 paradigm? Rather than using a microdose of the chemopreventive agent, can a microdose (i.e., <100 μg) of the carcinogen be administered? This design allows for the study of both the pharmacokinetics of the chemopreventive agent and its pharmacodynamic action on carcinogen disposition. Can this approach accelerate the development of protective interventions that reduce the mutagenesis and other forms of genetic damage imparted by exposures to environmental carcinogens? The primary considerations are technological—can we measure carcinogen levels after administering microdoses?—and ethical—can we safely administer carcinogen microdoses and obtain truly informed consent? We propose the label “reverse phase 0” for cancer prevention trials involving a microdose of a carcinogen.

Technology to Detect Microdose Levels

The available analytic technologies for therapy-drug phase 0 studies are constrained by the maximum allowable microdose of 100 μg , which is generally more than 100-fold lower than the doses used in traditional phase I pharmacology studies (19, 20), albeit within the upper bounds of most environmental exposures. The analytic constraints for prevention-related microdosing with carcinogens are more severe. In the two aflatoxin microdosing studies reported to date in people, the single-dose levels of aflatoxin B₁ were 1 μg (not given with a preventive agent; ref. 21) and 30 ng (15). This latter dose is likely comparable with that received by ingesting a peanut butter sandwich. Therefore, sensitive analytic technologies capable of measuring concentrations of either the parent compound or its metabolites at the nanogram-to-picogram or femtomole-to-attomole levels are needed to conduct this form of microdosing study. The technology must also exhibit specificity that limits the contribution of noise to the analysis. A desirable method would be very sensitive and have a large signal-to-noise ratio and a wide dynamic range; few technologies currently meet these very stringent criteria.

The many different analytic techniques that have been developed to measure parent compounds and their metabolites in human samples include the immunoassays ELISA and RIA; radiometric ³²P postlabeling method; and physicochemical methods UV-Vis or electrochemical detection, fluorescence spectroscopy, and mass spectrometry (22–31). Front-end separation methods such as gas chromatography and high-performance liquid chromatography have become very important for isolating individual compounds before final quantification. Separation technology has the additional advantage of lowering the contributions of noise to the analytic measurement.

Recent studies using liquid chromatography separation with isotope-dilution tandem mass spectrometry (LC-MS/MS) have shown a dramatic increase in sensitivity (32). Cur-

rently, the practical limit of LC-MS/MS detection is in the high-attomole to low-femtomole range for human biological samples, which is suitable for monitoring ambient carcinogen exposures from the environment, but may be currently too insensitive for carcinogen microdose studies. The accelerator MS (AMS) used in the Jubert et al. study measures the mass difference imparted by the incorporation of a ¹⁴C label into the test compound and has a detection limit of 1 adduct in 10¹² nucleotides (attomole to zeptomole range) and thus improves on that of LC-MS/MS (33–35). This limit places the measurement of pg/mL concentrations of analytes in biospecimens well within reach. However, Jubert et al. did not apply a separation technique before AMS measurements, and thus only total ¹⁴C (aflatoxin equivalents), not individual metabolites such as DNA adducts, was determined. It is important to note that radioactivity per se is not important to the measurement, rather the mass change is important. The amount of radiolytic decay that occurs in a microdosing experiment is of no health consequence relative to other everyday exposures to radiation.

Ethics in Phase 0 Human Microdosing Studies

Recent years have seen a number of commentaries and reviews on the design, structure, and ethical considerations inherent in phase 0 microdosing investigations in drug development (18, 36–38). In this context, the dose of 100 μg is typically less than 1/100th of a pharmacologically active level. As such, there is limited concern about safety, and only minimal toxicology studies are required before initiating a microdose study. The application of microdosing studies to environmental carcinogens has received far less attention. The availability of AMS, however, now makes investigating environmental-carcinogen kinetics, metabolism, and disposition practicable in humans. The primary question with respect to a microdose of an environmental carcinogen in people is whether the selected dose is biologically active. By definition, studies of microdoses of pharmacologically active agents are theoretically gated at dose levels far below an active dose based on animal data. There have been reports that this has not always been the case, illustrating the difficulty of extrapolating experimental animal data to humans (38). Not only experimental findings but, in many cases, also epidemiologic studies from high-risk human populations can provide dose-risk estimates for environmental carcinogens. For example, a microdose of 100 μg of aflatoxin B₁ is equivalent to the daily level of exposure to aflatoxin B₁ among the population at a high risk for hepatocellular carcinoma (39). Although virtually all environmental carcinogens require chronic exposure to affect disease outcomes, single exposures cannot be dismissed as being biologically inert. The aflatoxin B₁ microdosing levels used in people to date are from 100 to 3,300 times lower than the maximum microdose of 100 μg appropriate for a phase 0 study of a pharmaceutical agent. Carcinogen risk assessment models have no threshold risk, illustrating, theoretically at least, that some risk to the subject exists at any dose.

Fundamental differences between microdose investigations of pharmacologic agents and environmental carcinogens pose important issues for informed consent. There are well-established guidelines for informed consent of patients with disease enrolling in phase 1 clinical trials. There also has been substantial recent discussion about informed consent

for patients enrolling in phase 0 trials. Although the informed consent process for phase 0 trials of chemotherapeutic or other pharmacologically active agents undoubtedly will continue to evolve, this arena has at least been well vetted by institutional review boards. In studies of chemoprevention strategies for environmental carcinogens, such as the one published here, the major consideration is whether the dose compares with ambient environmental exposures. Risk communication is an essential component of the informed consent process. Participants, who will be healthy (noncancer but possibly high-risk) individuals or possibly presurgery resectable-cancer patients, need to be (a) informed that they are being exposed to a carcinogen; (b) provided a quantitative or at least qualitative estimate of their risk; and (c) advised that the risk is not offset by any direct benefit. These issues have been addressed by the Jubert et al. study, which was fully approved by its two institutional review boards.

Given the hypothesis, supported by prior studies, of the linearity of pharmacokinetic properties from active down to microdose ranges, initial exposures can be reduced to very low levels when highly sensitive analytic technologies such as AMS are used. With each individual serving as his or her own control, the numbers of people involved in these investigations are very low. As noted in Table 1, phase II trials of chemopreventive agents against environmental carcinogens typically enroll several hundred participants over durations of weeks or months, thus entailing a substantial burden of risk of adverse agent effects among a relatively large group of people. This burden is substantially mitigated or even possibly eliminated by the small size and short duration of a reverse phase 0 chemopreventive design.

An alternative to a microdose of an environmental carcinogen would be a microdose of an inactive analog of the carcinogen. For example, the congener aflatoxin B₂, which lacks the 8,9 double bond that yields the electrophilic epoxide, could be used as a surrogate for aflatoxin B₁. This surrogate might be perfectly adequate when the goal is to block bioavailability and uptake into the body. When identification and quantification of metabolites and adducts are necessary to fully understand efficacy and underlying mechanisms, the surrogate will be insufficient. Hecht and colleagues have used a surrogate approach with great success in administering microdoses of the noncarcinogenic PAH phenanthrene to evaluate interindividual variations in PAH metabolism and the effect of interventions seeking to alter PAH disposition (8, 40).

What Have Microdosing Studies Taught Us to Date?

Fewer than two dozen microdosing studies of pharmacologic agents have been published to date. They used drugs with previously established therapeutic doses and pharmacokinetic parameters, allowing a comparison between the microdosing pharmacokinetics and those of the established therapeutic dose or an assessment of the ability of the microdose studies to predict therapeutic-dose effects. As recently reviewed by Lappin and Garner (19), 15 of 18 drugs they examined showed linear pharmacokinetics within a factor of 2 between the microdose and the therapeutic dose, and thus, validation of microdose studies for evaluating drug pharmacokinetics is beginning to emerge. Only one previous report of a microdose of a carcinogen, aflatoxin B₁, has been published

(21). The study by Jubert et al. pushes this concept further by examining the effect of a pharmacologically relevant dose (150 mg) of the chemopreventive agent chlorophyllin or chlorophyll on the toxicokinetics of an aflatoxin microdose. Fortunately, it is possible to compare the results of this study with those of a previously completed phase II randomized, placebo-controlled, double-blind clinical trial of chlorophyllin in an eastern Chinese population at a high risk for exposure to aflatoxins and related liver cancer (9).

In the phase II trial, 100 mg of chlorophyllin three times a day for 3 months led to a 55% reduction (versus placebo) in the urinary excretion of the aflatoxin-DNA adduct aflatoxin-N⁷-guanine. Excreted DNA adduct levels were not measured at time points earlier than 3 months. The study size ($n = 200$) was driven largely by heterogeneity (established in previous exposure measurements; refs. 41, 42) in the ambient aflatoxin exposures of the study participants. The chlorophyllin dose was not selected by extrapolation of chemopreventive efficacy in animals but rather by the standard dose per labeling of commercially available chlorophyllin. Little safety data exist for this over-the-counter drug, although 50 years of use has not produced evidence of its toxicity. Reporting on three volunteers, Jubert et al. reached conclusions similar to those of the phase II study. The respective reductions among the three volunteers in the urinary excretion of aflatoxin equivalents (measured as a 0-to-24-hour area under the curve) were 58%, 28%, and 46% for chlorophyll and 0%, 17%, and 31% for chlorophyllin. The phase II and Jubert et al. studies are far from equivalent—a single, higher dose of the intervention (Jubert) compared with 270 doses (thrice daily for 3 months; phase II) and measures of all, nonspecified aflatoxin metabolites (Jubert) compared with measure of aflatoxin-N⁷-guanine (phase II). Nonetheless, would knowledge of the Jubert results have informed planning for the phase II chlorophyllin trial? Absolutely. It would have provided confidence that the selected phase II agent and dose would have a biological effect and would have suggested that a follow-up microdosing study to better establish dose-response relationships would be very informative. It also suggests that chlorophyll might be preferred over chlorophyllin, although the practicality of chlorophyll is questionable for long-term interventions (as discussed by Jubert et al.).

Certainly, Jubert et al. have validated the approach of using microdoses in prevention-related study of protection against an environmental carcinogen. Carcinogen candidates for further reverse phase 0 study might include aflatoxin, fumonisin, heterocyclic amines, and polycyclic aromatic hydrocarbons and other air toxins such as 1,3-butadiene and benzene (43). Candidate agent mechanisms for further reverse phase 0 assessments include modulation of absorption, inhibition of bioactivation, enhancement of detoxification and elimination, and induction of DNA repair capacity. We believe that chemopreventive phase 0 study is most appropriate for the setting of environmental carcinogenesis and agents potentially affecting it. As novel molecular targeted agents come online for prevention, the safety of the preventive agent in phase 0 testing also may come into play.

Chemoprevention researchers need to carefully consider interventions in populations at higher than de minimis risk for exposures to environmental carcinogens, carefully weighing insights into efficacy, safety (tolerability), cost, and practicality

in selecting the intervention agent. Microdosing experiments will not provide all the answers, nor are they intended to. For example, safety evaluations of chemopreventive agents will still reside largely in the domains of phase I, II, and III intervention trials. But as the development of therapeutic drugs might indicate, small, short, strategic microdosing trials can advance the prioritization of agent selections for chemopreventive interventions and of underlying mechanistic targets for further research. These trials also can better inform

the matching of agents to at-risk cohorts. In sum, microdosing studies with carcinogens have the potential to provide important insights into chemopreventive interventions and to enhance the overall clinical development and safety evaluation of preventive agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Hecht SS. Progress and challenges in selected areas of tobacco carcinogenesis. *Chem Res Toxicol* 2008;21:160–71.
- Knize MG, Felton JS. Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors in meat. *Nutr Rev* 2005;63:158–65.
- Groopman JD, Kensler TW, Wild CP. Protective Interventions to Prevent Aflatoxin-Induced Carcinogenesis in Developing Countries. *Annu Rev Public Health* 2008;29:187–203.
- Kelloff GJ, Boone CW, Crowell JA, et al. Risk biomarkers and current strategies for cancer chemoprevention. *J Cell Biochem Suppl* 1996;25:1–14.
- Lippman SM, Hawk ET. Cancer prevention: from 1727 to milestones of the past 100 years. *Cancer Res* 2009;69:5269–84.
- Hecht SS. Tobacco Smoke Carcinogens and Lung Cancer. *J Natl Cancer Inst* 1999;91:1194–210.
- Jacobson JS, Begg MD, Wang LW, et al. Effects of a 6-month vitamin intervention on DNA damage in heavy smokers. *Cancer Epidemiol Biomarkers Prev* 2000;9:1303–11.
- Kensler TW, Chen JG, Egner PA, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 2005;14:2605–13.
- Egner PA, Wang JB, Zhu YR, et al. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. *Proc Natl Acad Sci U S A* 2001;98:14601–6.
- Wang JS, Shen X, He X, et al. Protective alterations in phase 1 and 2 metabolism of aflatoxin B₁ by oltipraz in residents of Qidong, People's Republic of China. *J Natl Cancer Inst* 1999;91:347–54.
- Luo H, Tang L, Tang M, et al. Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. *Carcinogenesis* 2006;27:262–8.
- Wang P, Afriyie-Gyawu E, Tang Y, et al. NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: II. Reduction in biomarkers of aflatoxin exposure in blood and urine. *Food Addit Contam* 2008;25:622–34.
- Kensler TW, Groopman JD, Roebuck BD. Use of aflatoxin adducts as intermediate endpoints to assess the efficacy of chemopreventive interventions in animals and man. *Mutat Res* 1998;402:165–72.
- Kensler TW. Chemoprevention by inducers of carcinogen detoxication enzymes. *Environ Health Perspect* 1997;105 Suppl 4:965–70.
- Jubert C, Mata JE, Bench G, et al. Effects of chlorophyll and chlorophyllin on low-dose aflatoxin B₁ pharmacokinetics in human volunteers. *Cancer Prev Res* 2009;2:1015–22.
- Dashwood R, Negishi T, Hayatsu H, Breinholt V, Hendricks J, Bailey G. Chemopreventive properties of chlorophylls towards aflatoxin B₁: a review of the antimutagenicity and anticarcinogenicity data in rainbow trout. *Mutat Res* 1998;399:245–53.
- Bielas JH, Loeb LA. Mutator phenotype in cancer: timing and perspectives. *Environ Mol Mutagen* 2005;45:206–13.
- Kinders R, Parchment RE, Ji J, et al. Phase 0 clinical trials in cancer drug development: from FDA guidance to clinical practice. *Mol Interv* 2007;7:325–34.
- Lappin G, Garner RC. The utility of microdosing over the past 5 years. *Expert Opin Drug Metab Toxicol* 2008;4:1499–506.
- Garner RC, Lappin G. The phase 0 microdosing concept. *Br J Clin Pharmacol* 2006;61:367–70.
- Cupid BC, Lightfoot TJ, Russell D, et al. The formation of AFB₁-macromolecular adducts in rats and humans at dietary levels of exposure. *Food Chem Toxicol* 2004;42:559–69.
- Wogan GN. Molecular epidemiology in cancer risk assessment and prevention—recent progress and avenues for future research. *Environ Health Perspect* 1992;98:167–78.
- Wogan G. Markers of exposure to carcinogens. *Environ Health Perspect* 1989.
- Weston A. Physical methods for the detection of carcinogen-DNA adducts in humans. *Mutat Res* 1993;288:19–29.
- Wang JS, Groopman JD. Biomarkers for carcinogen exposure: tumor initiation Molecular Biology of the Toxic Response. Washington (DC): Taylor & Francis; 1998.
- Strickland PT, Routledge MN, Dipple A. Methodologies for measuring carcinogen adducts in humans. *Cancer Epidemiol Biomarkers Prev* 1993;2:607–19.
- Santella RM. Immunological methods for detection of carcinogen-DNA damage in humans. *Cancer Epidemiol Biomarkers Prev* 1999;8:733–9.
- Randerath K, Reddy MV, Gupta RC. P-32-Labeling Test for DNA Damage. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences 1981;78:6126–9.
- Randerath K, Randerath E. P-32 postlabeling methods for DNA adduct detection—overview and critical evaluation. *Drug Metab Rev* 1994;26:67–85.
- Phillips DH, Farmer PB, Beland FA, et al. W. Methods of DNA adduct determination and their application to testing compounds for genotoxicity. *Environ Mol Mutagen* 2000;35:222–33.
- Kaderlik RK, Lin DX, Lang NP, Kadlubar FF. Advantages and limitations of laboratory methods for measurement of carcinogen-DNA adducts for epidemiological studies. *Toxicol Lett* 1992;64–65 Spec No:469–75.
- Egner PA, Groopman JD, Wang JS, Kensler TW, Friesen MD. Quantification of aflatoxin-B₁-7-Guanine in human urine by high-performance liquid chromatography and isotope dilution tandem mass spectrometry. *Chem Res Toxicol* 2006;19:1191–5.
- Dingley KH, Curtis KD, Nowell S, Felton JS, Lang NP, Turteltaub KW. DNA and protein adduct formation in the colon and blood of humans after exposure to a dietary-relevant dose of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Cancer Epidemiol Biomarkers Prev* 1999;8:507–12.
- Vogel JS, Turteltaub KW, Finkel R, Nelson DE. Accelerator mass spectrometry. *Anal Chem* 1995;67:353–9A.
- Hah SS. Recent advances in biomedical applications of accelerator mass spectrometry. *J Biomed Sci* 2009;16:54.
- LoRusso PM. Phase 0 clinical trials: an answer to drug development stagnation? *J Clin Oncol* 2009;27:2586–8.
- Abdoler E, Taylor H, Wendler D. The ethics of phase 0 oncology trials. *Clin Cancer Res* 2008;14:3692–7.
- Kimmelman J. Ethics at phase 0: clarifying the issues. *J Law Med Ethics* 2007;35:727–33, 514.
- Groopman JD, Jiaqi Z, Donahue PR, et al. Molecular dosimetry of urinary aflatoxin-DNA adducts in people living in Guangxi Autonomous Region, People's Republic of China. *Cancer Res* 1992;52:45–52.
- Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 2002;23:907–22.
- Wang JS, Qian GS, Zarba A, et al. Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 1996;5:253–61.
- Kensler TW, He X, Otieno M, et al. Oltipraz chemoprevention trial in Qidong, People's Republic of China: modulation of serum aflatoxin albumin adduct biomarkers. *Cancer Epidemiol Biomarkers Prev* 1998;7:127–34.
- Kensler TW, Wakabayashi N. Nrf2: friend or foe for chemoprevention? *Carcinogenesis*. 2009 Sep 30. [Epub ahead of print].
- Camoirano A, Bagnasco M, Bennicelli C, et al. Oltipraz chemoprevention trial in Qidong, People's Republic of China: results of urine genotoxicity assays as related to smoking habits. *Cancer Epidemiol Biomarkers Prev* 2001;10:775–83.
- Glintborg B, Weimann A, Kensler TW, Poulsen HE. Oltipraz chemoprevention trial in Qidong, People's Republic of China: unaltered oxidative biomarkers. *Free Radic Biol Med* 2006;41:1010–4.
- Tang L, Tang M, Xu L, et al. Modulation of aflatoxin biomarkers in human blood and urine by green tea polyphenols intervention. *Carcinogenesis* 2008;29:411–7.
- Johnson NM, Afriyie-Gyawu E, Huebner H, et al. PAH exposure in a Ghanaian population at high risk for aflatoxicosis. *Sci Total Environ* 2009;407:1886–91.