

## EXTENDED ABSTRACTS

### Proceedings of the American Statistical Association 2000 Conference on Radiation and Health

Park City, Utah, June 25–28, 2000

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## A Brief History of the American Statistical Association Conference on Radiation and Health

Jay H. Lubin

*National Cancer Institute, Bethesda, Maryland*

The conference in Park City, UT was the 14th in a series of conferences beginning in 1981, sponsored by the American Statistical Association (ASA), on the health effects of radiation exposures. The initial organizing committee [including Drs. Peter Lachenbruch (chair), Robert Wallace, Bernard Pasternack, Charles Lynch, R. L. Anderson (representing the ASA) and C. Spector (representing the Environmental Protection Agency)] proposed a yearly meeting for the purpose of “bring[ing] together statisticians and epidemiologists to discuss various problems in the analysis of the biological effects of ionizing radiation.” At the first conference, there were about equal numbers of statistical and epidemiological presentations. Under the guidance of the 1982 organizing committee [including Drs. Bernard S. Pasternack (chair), Theodore Kneip, Peter Lachenbruch, Howard Greenberg, R. L. Anderson (ASA), and R. Brandwein (EPA)] and the 1983 organizing committee [including Drs. Ray A. Waller (chair), Edward L. Frome, Ethel S. Gilbert, Bernard S. Pasternack, Gary L. Tietjen, R. L. Anderson (ASA), and Fred C. Leone (ASA)], the objectives of the conference broadened to “facilitate effective communication and technical interaction among environmental scientists, epidemiologists, health scientists, life scientists, physical scientists, and statisticians to address problems related to ionizing radiation and associated environmental and health issues.” In subsequent years, the objectives were further enlarged to include effects of ionizing and non-ionizing radiation in cellular, experimental animal and human systems, and risk assessment, as well as a variety of statistical issues, such as dose-response modeling, low-dose risk estimation, meta-analysis methods, biologically motivated risk models, study design, and the consequences of exposure measurement error.

From the outset, the intention of the organizers was a relatively small (50–80 presenters and attendees), multidisciplinary, Gordon-type conference that met in a semi-isolated location. There would be no concurrent sessions. Presentations would be comprehensive, with a format that would allow an extensive discussion period, so that topics would be critically evaluated. In addition, presentations were limited to mornings and evenings, so that participants had the opportunity for substantial and informal interactions outside the scientific sessions.

For the first 7 years from 1981–1987, the conference was held in Coolfont, WV, and became known as the “Coolfont Conference”. The themes of the first two conferences were “Statistical and Epidemiological Methods in Ionizing Radiation” and “Environmental Sampling and Analysis of Sampling Data: Assessment of Human Exposures and Health Effects”, respectively. From 1983, Coolfont conferences had no specifically designated themes, but topics were structured under the general heading “Radiation and Health”, which became the conference appellation.

Yearly conferences became unsustainable, and the eighth conference was held in 1989. The conference was reorganized, becoming biennial starting in 1990 and more thematic, and held at a variety of venues. Starting in 1989, the next seven meetings (with dates and themes) were held in Copper Mountain, CO (1989: “Health Effects of Electric and Magnetic Fields: Statistical Support for Research Strategies”; and 1990: “Ionizing Radiation Risks: Present and Future”), Hilton Head, SC (1992: “Radiation Risk and Interactions”), Nantucket, MA (1994: “Dosimetry and Risk Assessment”), Vail, CO (1996: “Radiation Risk Assessment, Statistical Methodology and Mechanisms”), San Diego, CA (1998: “Radiation Effects at Low Doses”), and Park City, UT (2000: “Temporal Factors and Radiation Effects”).

At the 1981 conference, participants identified a variety of topics of scientific importance. Topics included biomathematical modeling, epidemiological designs, time-varying dosages, communication to the public, overview sessions, low-dose extrapolation, estimation of latent periods, and handling missing data. These topics highlighted the major areas of radiation research at that time and became the focus of many sessions.

Currently, the conference attempts to include the full spectrum of radiation-related research topics.

As expected from a conference spanning 20 years, topics have frequently been revisited, so that the newest information can be presented. For example, studies among the Japanese atomic bomb survivors have been the subject of many sessions, highlighting the central role of this population in radiation research, and epitomizing the changing scientific interests. At the 1981 conference, the atomic bomb survivors were the subject of talks on methodology (Gilbert Beebe), low-dose modeling (Charles Land), dosimetry (George Kerr), non-carcinogenic effects (Seymour Jablon), and human genetic effects (William Schull). Analytical methodology for the Life Span Study cohort of atomic bomb survivors was revisited in 1983 (Donald Pierce), while dosimetry issues were revisited in 1985 (George Kerr) and 1986 (William Ellett). As additional follow-up has accrued, radiation disease effects have been updated and expanded (1985: radiation and smoking, William Blot; 1990: modeling disease incidence, Dale Preston; 1992: incidence of solid tumors, Elaine Ron; 1992: neutrons and uncertainties in risk estimates, Seymour Jablon; 1994: *in utero* exposure effects, William Schull; 2000: biologically motivated models, Donald Pierce and Suresh Moolgavkar; 2000: non-cancer outcomes, Dale Preston). Similarly, exposure to radioactive radon gas in mines and in houses has been a continuing topic. There have been presentations on effects of exposure to radon and its decay products in 11 of the 14 conferences, highlighting the scientific evolution of radon effects over the past two decades and its importance as the principal component of radiation exposure in the general population.

Summary reports from the earliest conferences suggested a tension between the needs of scientists and “basic science” and the requirements of “regulators”. Although the focus of the conference has always been scientific, topics were selected that acknowledged the needs of regulatory agencies. In addition to a continuing emphasis on low-dose modeling of disease risk and several presentations on adaptive response to radiation exposure, topics which have both scientific and regulatory implications, there have been a variety of regulatory-related presentations (1982: environmental monitoring of exposures, speaker not identified in the program; 1987: national radon risk assessment, Richard Guimond; 1990: environmental transport models, Steven Bartell; 1992: issues in risk assessment, Ethel Gilbert; 1996: identifying areas of high residential radon concentrations, Anthony Nero; 1983: risk projection models, Naomi Harley; and 1984, 1985, 2000: probability of causation, Seymour Jablon, Frederick Mosteller and Charles Land, and Duncan Thomas and Sander Greenland, respectively). There have also been presentations on the history of radiation protection (1987, Edward Pochin), the cost of residential radon mitigation (1992, Daniel Krewski), and the incorporation of radiosensitive subpopulations in radiation risk assessment (1998, R. Julian Preston).

A particular strength of continuing conferences has been the opportunity for a wide variety of tutorials and special topics that enhance communication across disciplines. In the 14 ASA conferences to date, there have been special presentations on a variety of topics, including rate standardization, analysis of sampling data, risk assessment, spatial and robust statistics, analysis of cohort studies, probability of causation, the measurement and potential effects of electric and magnetic fields, effects of errors in dosimetry, multiple comparisons, meta-analysis, Bayesian methods for combining studies, mechanisms of carcinogenesis, experimental animal studies, radiation cytogenetics, biodosimetry and molecular signatures.

If history is a guide, then this conference will continue to highlight the most important challenges facing radiation health effects research. The 14th ASA Conference on Radiation and Health included presentations on the consequences of, and methods of adjustment for, exposure measurement error, the application of biologically motivated multistage models to epidemiological data, temporal effects in radiation epidemiology, dose and dose-rate effects at the cellular and epidemiological levels, radiosensitivity, susceptible subpopulations, and the evolving interrelationship of molecular biology and human genetic epidemiology. As the study of radiation effects continues, increasingly greater emphasis will be placed on

linking biological parameters and processes and molecular pathways directly to data on human disease outcome, and on the consequences of radiation and environmental exposures and the role of specific genetic polymorphisms. The range of radiation doses of greatest interest to the general population is precisely the range where epidemiology provides the least assistance. As a result, there will be an increasing reliance on molecular and radiobiology to bridge this gap and provide greater confidence in, and scientific support for, low-dose risk estimates.

Finally, on a practical level, the conference has been fortunate in its ability to maintain a relatively stable funding base from a variety of governmental and non-governmental organizations. However, adequate funding is vital to maintain a full conference schedule, as well as expand the scientific base to include a greater number of international colleagues and create opportunities for the participation of young researchers through grants supporting travel and registration. Scientifically, the conference will continue, as in the past, to enhance and expand its interdisciplinary focus, providing programs at the cutting edge of radiation science and fostering new directions and new collaborations.

## I. DEBATES

### Resolved: Biologically Based Models are Useful for Analyzing Radiation Epidemiological Data

Chair: Evan Douple

*National Academy of Sciences, Washington, D.C.*

**PRO:** Daniel Krewski, *University of Ottawa, Ottawa, Ontario, Canada*

Both empirical (1) and biologically based (2) models have found application in assessing the risks of cancer-causing substances in the environment. Empirical models are effectively statistical models that provide a parsimonious description of the available data without consideration of biological mechanisms of action. Biological models, on the other hand, are based on a plausible biological theory of the fundamental biological events such as mutation and cell proliferation involved in neoplastic transformation.

Biological models have a number of advantages over empirical models in cancer risk assessment (3). First, because biological models have a mechanistic basis, they may enjoy greater acceptance than empirical models, particularly if the model incorporates those events known to play a role in neoplastic conversion in a meaningful way. Second, the use of biologically based models invites meaningful questions that may stimulate further research to deepen our understanding of the process of carcinogenesis as an incomplete or inadequate model is first rejected and then refined. Third, the parameters of a biologically based model afford a direct biological interpretation, and they can be examined for biological plausibility as part of the process of model validation. Fourth, a validated biologically based model may lead to more accurate predictions of risk in different species in cases where accurate estimates of those parameters that are species specific are available. Fifth, extrapolation from high to low doses may be done more accurately and with greater confidence using a validated biologically based model, since mechanisms of carcinogenesis are not likely to differ qualitatively at different dose levels. And sixth, biologically based models may provide a basis for better characterizations of uncertainty associated with cancer risk predictions. Although the development of a biologically based model may require more effort and data than an empirical risk model, the potential for more accurate predictions of risk can lead to more appropriate risk management decisions.

Radon, which has been shown to interact in a synergistic manner with tobacco smoke in the induction of lung cancer in underground miners, can be described using the two-stage clonal expansion model of carcinogenesis (4, 5). This biologically based model presumes that two critical mutations are required to convert a stem cell to a malignant state, and that the size of the pool of intermediate cells that have sustained the first

mutation may be increased by promoting agents that selectively increase the rate of proliferation of intermediate cells. Analyses of data on the Colorado uranium miners suggest that radon acts both as an initiator, increasing the first-stage mutation rate, and as a promoter. However, application of the two-stage model to a cohort of Chinese tin miners exposed to radon suggests that the second-stage mutation rate may also be affected (6). The two-stage model has also been used successfully to describe temporal patterns of cancer risk among atomic bomb survivors (7). Because the radiation exposure was instantaneous, only initiation was assumed to be affected by exposure.

The two-stage model predicts both the inverse dose-rate effect in miners exposed to radon and the direct dose-rate effect among A-bomb survivors. The former effect, which is negligible at low doses, is attributed to increased cell proliferation after protracted radon exposure, whereas the latter effect is associated with initiation. The ability to distinguish quantitatively between the effects of initiation and promotion at different dose levels is an important property of the two-stage model. This distinction was pivotal in a recent regulatory application of the two-stage model in describing the dose-response curve for squamous cell carcinomas of the nasal passage of rats exposed to formaldehyde (8), in which the promotional effects of formaldehyde appeared to be the most important determinant of cancer risk.

Although cancer risk estimates are subject to uncertainty (9), the applications noted above indicate that biologically based risk models are useful in radiation risk assessment. Biologically based models permit incorporation of time-dependent exposure patterns in risk modeling in a natural way and provide a basis for interpreting temporal characteristics such as the inverse and direct dose-rate effects seen with high- and low-LET radiation, respectively. Although both biologically based and flexible empirical models can provide good fits to most data sets, careful construction, validation and interpretation of biologically based models can provide valuable insights into the mechanisms of radiation carcinogenesis.

### References

1. D. Krewski, E. Cardis, L. Zeise and V. J. Feron, Empirical approaches to risk estimation. In *Quantitative Estimation and Prediction of Human Cancer Risks* (S. H. Moolgavkar, D. Krewski, L. Zeise, E. Cardis and H. Moller, Eds.), pp. 131–178. Scientific Publications No. 131, IARC, Lyon, 1999.
2. S. H. Moolgavkar, M. Schwarz and D. Krewski, Mechanisms of carcinogenesis and biologically based models for the estimation and prediction of risk. In *Quantitative Estimation and Prediction of Human Cancer Risks* (S. H. Moolgavkar, D. Krewski, L. Zeise, E. Cardis and H. Moller, Eds.), pp. 179–237. Scientific Publications No. 131, IARC, Lyon, 1999.
3. M. J. Goddard and D. Krewski, The future of mechanistic research in risk assessment: Where are we going and can we get there from here? *Toxicology* **102**, 53–70 (1995).
4. S. H. Moolgavkar, E. G. Luebeck, D. Krewski and J. Zielinski, Radon, cigarette smoke and lung cancer: An analysis of the Colorado plateau uranium miners' data. *Epidemiology* **4**, 204–217 (1993).
5. E. G. Luebeck, W. F. Heidenreich, W. D. Hazelton, H. G. Paretzke and S. H. Moolgavkar, Biologically based analysis of the data for the Colorado uranium miners cohort: Age, dose and dose-rate effects. *Radiat. Res.* **152**, 339–351 (1999).
6. E. G. Luebeck, The importance of promotion in lung carcinogenesis for protracted exposures to radon and radon progeny. *Radiat. Res.* **154**, 730–731 (2000). [extended abstract]
7. M. Kai, E. G. Luebeck and S. H. Moolgavkar, Analysis of the incidence of solid tumors among atomic bomb survivors using a two-stage model of carcinogenesis. *Radiat. Res.* **148**, 348–358 (1997).
8. Health Canada, *Priority Substances List Assessment Report: Formaldehyde*. Healthy Environments and Consumer Safety Program, Ottawa, in press.
9. D. Krewski, S. N. Rai, J. M. Zielinski and P. K. Hopke, Character-

ization of uncertainty and variability in residential radon risks. *Ann. NY Acad. Sci.* **895**, 245–272 (1999).

**CON:** Kenny S. Crump, *ICF Consulting, Ruston, Louisiana*

The term “biologically based model” refers to a model whose parameters have physiological meaning and could, at least in theory, be measured by direct experimentation. Examples of such models include pharmacokinetic models of the distribution and metabolism of chemicals within the body (1) and the two-stage model of cancer (2). The U.S. EPA (3) draft guidelines for cancer risk assessment defined a biologically based model for cancer very stringently as one “whose parameters are calculated independently of curve-fitting of tumor data”.

#### Potential Uses

There are several reasons for applying a model to epidemiological data, including:

1. To determine if an effect is present and estimating its magnitude.
2. To make predictions outside of the region of observation.
3. To understand mechanisms.

In item 1, the crucial issues are confounding, statistical power, and precision of data. Although some confounding variables (e.g. age, other exposures) may be incorporated naturally into a biologically based model, others (e.g. socioeconomic) will need to be handled in much the same manner as in standard statistical models. In many cases, a biologically based model may add little to such an analysis, and the added complexity of such a model may obfuscate rather than enlighten.

Extrapolations beyond the range of the data include extrapolation to different temporal exposure patterns, to younger or older ages, to different routes of exposure, and to lower doses. Biologically based models have obvious advantages over statistical models in such applications. A pharmacokinetic model can be used to predict exposures at critical target tissues from both air and oral routes of exposure and the result can be used to estimate the risk of oral exposure from a study of air exposures. A biologically based model of cancer, possibly coupled with a pharmacokinetic model, can facilitate extrapolation from short-term exposures to lifetime exposures.

Biologically based models also have a distinct advantage over statistical models in understanding mechanism of action. Predictions from models incorporating specific mechanisms of action can be compared to data and used to test hypotheses regarding these mechanisms.

#### Potential Concerns

However, there are a number of potential concerns regarding these applications of biologically based models. The term “biologically based model” has a very intuitive appeal to biologists and other nonstatisticians, who may already be suspicious of outputs from more standard statistical models. As a result, they may tend to accept results from a biologically based model without fully understanding its assumptions or limitations. It is therefore particularly important for modelers to be very explicit about the assumptions and limitations of these models.

Some applications may incorporate parameter estimates (e.g. estimates of metabolic rates or cell proliferation rates) obtained from data in ancillary experiments. The relevance of such data to the particular epidemiological population of interest may be questionable. Because of lack of data for humans, some parameters may have to be estimated from non-human data. Even if data for humans are available, they may have been obtained from a non-representative population. Measured cell division rates for use in a biologically based model of cancer may not be from the cells most at risk of progressing to cancer. Even if the ancillary data used in a biologically based model are representative, there may be large statistical uncertainty in the output stemming from uncertainties in each of numerous estimated parameters.

It must be clear what the limitations of the model are. The two-stage model of cancer (2) expresses the probability of cancer as a function of rates of cell division, death and mutation. To enhance mathematical trac-

tability, simplifying assumptions are made that include independent action of cells, unregulated clonal growth, and exponentially distributed cell lifetimes. Since these assumptions may not be realistic, it is important that conclusions based on the model not be sensitive to these assumptions. The model does not directly incorporate the effect of dose, and a dose effect is normally added by simply assuming a dose response for one or more of the underlying parameters. Although the resulting dose response for cancer can be compared to data, unless the assumed dose responses for the more basic parameters have some valid biological basis, predictions of the effects of low doses will be subject to the same uncertainties as those made from purely statistical models (4, 5).

#### References

1. H. J. Clewell and M. E. Andersen, Dose, species and route extrapolation using physiologically based pharmacokinetic models. In *Drinking Water Health*, Vol. 8, pp. 159–182. National Research Council, Washington, DC, 1987.
2. S. Moolgavkar and G. Luebeck, Two-event model for carcinogenesis: Biological, mathematical, and statistical considerations. *Risk Anal.* **10**, 323–341 (1990).
3. *Proposed Guidelines for Carcinogen Risk Assessment*. EPA/600/P-92/003C, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC, 1996.
4. K. Crump, Limitations of biological models of carcinogenesis for low-dose extrapolation. *Risk Anal.* **14**, 883–886 (1994).
5. K. Crump, Use of mechanistic models to estimate low dose cancer risks. *Risk Anal.* **14**, 1033–1038 (1994).

### Resolved: The Probability of Causation can be Used in an Equitable Manner to Resolve Radiation Tort Claims and Design Compensation Schemes

Chair: Robert Rinsky

*National Institute for Occupational Safety and Health, Cincinnati, Ohio*

**PRO:** Duncan C. Thomas, *University of Southern California, Los Angeles, California*

The probability of causation (PC) is defined as the probability that a particular exposure caused or contributed to the development of disease in an affected individual with that exposure. A widely used estimator of this quantity from epidemiological data is  $(r - 1)/r$ , where  $r$  is the estimated relative risk associated with a case's exposure history. This calculation underlies the “Radioepidemiologic Tables” (1) which have been used to resolve the claims of the atomic veterans and others. In a commentary on these tables, the NAS Oversight Committee (2) pointed out that these were not strictly probabilities and suggested the term “Assigned Shares” instead. Considering that the Radioepidemiologic Tables are currently being updated, it is timely to revisit some of the fundamental issues that have been raised about their interpretation and how uncertainties in PC estimates ought to be incorporated into decision-making.

In a series of papers, Robins and Greenland (3, 4) have argued that the PC is not estimable for individuals without making unverifiable assumptions concerning biological mechanisms and the extent of heterogeneity between individuals. In particular, they have demonstrated scenarios in which the relative risk could be arbitrarily close to 1 (and hence the PC would be close to zero), yet all cases were affected by exposure in the sense that their death times had been advanced by some small amount. They have also shown that even the population mean of a heterogeneous distribution of PCs is not estimable and that the compensation schemes that pay in proportion to the PC are neither “robust” to model mis-specification nor “economically rational” in the sense of assessing total damages in proportion to the amount of harm caused; however, they have also shown that robust and rational compensation schemes can be developed based on expected loss of life expectancy.

These points are well taken and mathematically correct. I have no dispute with these authors about the inherent uncertainty in PC estimates and the importance of allowing for these uncertainties in reaching compensation decisions or about the bias in PC estimates that can be caused by heterogeneity in baseline rates. I also agree that compensation schemes based on loss of life expectancy are more appropriate than those based solely on the PC and that schemes that pay in proportion to either quantity are more rational than all-or-none schemes based solely on whether the PC estimate is greater than 50% or not. However, I disagree that these theoretical problems of estimability invalidate the utility of the PC for decision-making and feel that realistic degrees of heterogeneity produce only moderate bias. In particular, I shall show that, despite the overall underestimation in absolute PCs, this quantity still provides a good basis for approximately ranking claims.

Following Robins and Greenland (3, 4), let  $\lambda_i(t, Z)$  denote the hypothetical hazard rate that individual  $i$  would experience were he exposed to  $Z$ . Then the individual's probability of causation is  $p_i = [\lambda_i(t, Z) - \lambda_i(t, 0)] / \lambda_i(t, Z)$ . Of course, these individual hazard functions are not directly observable, and hence neither is  $p_i$ . The population rate is given by  $\lambda_*(t, Z) = E[\lambda_i(t, Z)] = \sum_i \lambda_i(t, Z) S_i(t, Z) / \sum_i S_i(t, Z)$ , where  $S_i(t, Z)$  is the corresponding individual survival function. The "naive PC" (or "rate fraction" in the terminology of Greenland and Robins) is given by  $p_* = [\lambda_*(t, Z) - \lambda_*(t, 0)] / \lambda_*(t, Z)$ , which in general will not equal  $E(p_i)$  if there is heterogeneity between individuals in their baseline hazards. In fact, it can be shown that  $p_* \leq E(p_i)$ .

How much heterogeneity is it reasonable to expect might actually exist? Unfortunately, for a non-recurrent event like death, survival times of independent individuals provide no information about the variability in individual hazard rates. However, such information can be obtained from a study of related individuals, particularly monozygotic twins, who are perfectly matched on genotype and tend to have experienced similar environments (identical *in utero* and very similar childhood). Frailty models can be used to estimate the variance *between* twin pairs in the component of the baseline risks they share. This can be interpreted as an estimate of the variance in baseline risks between unrelated individuals.

The best data on lifetime survival data in twins come from the registry of Danish twins born from 1881–1930. These data have been fitted using a variety of models, of which I will focus on the gamma-frailty model,  $\lambda_i(t, Z) = \lambda_0(t) X_i r(Z_{ij})$ , where the frailty  $X_i$  shared by twin pair  $i$  is assumed to follow a gamma distribution with variance  $1/\theta$  and  $r(Z_{ij})$  is the relative risk for member  $j$  of the pair. Hougaard *et al.* (5) provide an estimate of  $\hat{\theta} = 1.94$  (SE 0.32) in males and 2.11 (SE 0.42) in females, much lower than for dizygotic twins (5.65 and 3.49, respectively) using a nonparametric baseline hazard. Anderson *et al.* (6) provide a similar estimate of 2.55 in monozygotic twins (compared with 8.99 in dizygotic twins) using a parametric Gompertz baseline hazard. To illustrate the implications for between-individual heterogeneity in baseline risks, I will adopt a value of  $\theta = 2$  in a proportional hazards frailty model with a Weibull baseline risk with exponent  $k = 6$  and a relative risk of 2. This corresponds to a 10th–90th percentile interval of frailties  $X_i$  from 0.26 to 1.93, a range of more than 7-fold. The true PC,  $p_i$ , is of course exactly 0.5 for all individuals in this model. Up to about age 50 (90% survival), there is no noticeable bias in the naive estimate  $p_*$  based on the marginal hazard rate; thereafter, the naive PC declines to about 0.2 by age 90 (10% survival), reflecting the effect of differential survival—high-frailty individuals in an exposed population are being eliminated faster than their counterparts in an unexposed population.

Greenland and Robins have also discussed models in which all individuals' death times are advanced by an amount that depends upon exposure. This can be expressed in terms of the "accelerated failure time model", in which  $S_i(t, Z) = S_{0i}(t - Z\beta)$ , where  $S_{0i}(t)$  is, for argument's sake, the survival curve derived from the same gamma-frailty model discussed above. Similar calculations based on an assumption of  $\theta = 2$  and an acceleration of 2 years for all subjects produce a pattern of declining PCs with increasing age, whether computed as  $p_*$  or  $p_i$ , although again the naive PC begins to underestimate the true PC starting at about age 50. In particular, note that the true PC is less than 100% in this frame-

work, even though we have assumed that the entire survival distribution has been shifted by a constant 2 years.

Despite these biases due to differential survival, the strongest determinants of an individual's PC are still his dose and age at death, so it is reasonable to inquire whether the naive PC could still be used to rank individuals. To investigate this question, I computed the naive  $p_*$  and the true  $p_i$  for 450 combinations of  $X_i$ ,  $Z_i$  and  $t_i$ , chosen to represent a reasonable range of possibilities.  $X_i$  were chosen from the 10th, 30th, 50th, 70th and 90th percentiles of a gamma distribution with  $\theta = 2$ ; 10 values of  $Z_i\beta$  were chosen to produce a range of PCs from 10% to 90%; and for each combination of  $X_i$  and  $Z_i\beta$ , 9 values of  $t_i$  were selected from the 10th to the 90th percentiles of the corresponding distributions among cases. Although there was some underestimation of the true PC by the naive  $p_*$  for many of these scenarios, the overall correlation between  $p_*$  and  $p_i$  was 0.995 under the accelerated failure time model and 0.969 under the conditional proportional hazards model. Since, as Robins and Greenland have pointed out, the calculation of the PC depends upon unverifiable assumptions of model, I also computed  $p_*$  under a mis-specified constant relative risk model where the true model was the accelerated failure time model; the correlation was still 0.971. I therefore conclude that the naive PC can indeed be used to approximately rank an individual's claims.

The "balance of probabilities" principle in tort law has been widely interpreted by the courts as requiring that the point estimate of the PC be at least 50%. If these estimates are inherently uncertain, then even setting aside the questions of bias discussed above, it is evident that some individuals whose PC estimates are just below 50% will be unjustly denied compensation (and vice versa). To allow for this, some agencies such as the Veterans Administration have taken a more liberal approach to triaging claims by considering all those for whom the upper 99% confidence limit on the PC is at least 50%. Unfortunately, this approach has the unsatisfactory feature of favoring claimants for whom the evidence of a causal association is the weakest. For example, a claim with a PC of 45% with confidence limits 42–48% would lose, while one with a PC of 10% and confidence limits 0–90% would win, even though there is much stronger evidence of a population association for the former than the latter. More rational strategies might pay in proportion to the expected value of the PC, or the posterior probability that the PC is greater than 50%.

#### References

1. Report of the National Institutes of Health Ad Hoc Working Group to Develop Radioepidemiologic Tables. Publication No 85-2748, National Institutes of Health, Washington, DC, 1985.
2. S. W. Lagakos and F. Mosteller, Assigned shares in compensation for radiation-related cancers. *Risk Anal.* **6**, 345–357 (1986).
3. J. Robins and S. Greenland, The probability of causation under a stochastic model for individual risk. *Biometrics* **45**, 1125–1138 (1989).
4. J. Robins and S. Greenland, Estimability and estimation of expected years of life lost due to a hazardous exposure. *Stat. Med.* **10**, 79–93 (1991).
5. P. Hougaard, B. Harvald and N. V. Holm, Measuring the similarities between the lifetimes of adult Danish twins born between 1881–1930. *J. Am. Stat. Assoc.* **87**, 17–24 (1992).
6. J. E. Anderson, T. A. Louis, N. V. Holm and B. Harvald, Time-dependent association measures for bivariate survival distributions. *J. Am. Stat. Assoc.* **87**, 641–650 (1992).

CON: Sander Greenland, UCLA School of Public Health and Department of Statistics, Los Angeles, California

The concept of probability of causation forms the basis of important legal standards, legislation and compensation schemes, which in turn use epidemiological data to estimate the probability of causation by equating the latter to the attributable fraction. This usage is a misapplication of epidemiology, for it has been shown that epidemiological data cannot

supply estimates of the probability of causation without imposing restrictive biological assumptions (1–5). The continued misapplication of probability-of-causation concepts stems from a need to resolve cases in a rational and consistent manner. This need does not, however, justify the continued misuse of epidemiological data in compensation decisions. Compensation schemes and legal standards need to recognize that an upper bound on the probability of causation cannot be determined from epidemiological data alone; biological models are also needed. Although equitable compensation schemes can be formulated without reference to the probability of causation, all schemes must deal with fundamental methodological uncertainties in estimation.

#### References

1. L. A. Cox, Probability of causation and the attributable risk. *Risk Anal.* **4**, 221–230 (1984).
2. J. M. Robins and S. Greenland, The probability of causation under a stochastic model for individual risks. *Biometrics* **45**, 1125–1138 (1989).
3. S. Greenland, The relation of the probability of causation to the relative risk and the doubling dose: A methodologic error that has become a social problem. *Am. J. Pub. Health* **89**, 1166–1169 (1999).
4. J. Beyea and S. Greenland, The importance of specifying the underlying biologic model in estimating the probability of causation. *Health Phys.* **76**, 1–6 (1999).
5. S. Greenland and J. M. Robins, Epidemiology, justice, and the probability of causation. *Jurimetrics* **40**, 321–340 (2000).

## II. UNCERTAINTIES IN EXPOSURE AND IN RESPONSE

Chair: E. Vincent Holahan

*Nuclear Regulatory Commission, Washington, D.C.*

### Uncertainties in Medical Radiation Exposures

H. D. Royal

*Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri*

Diagnostic medical radiation exposures are the largest contributor to man-made sources of radiation exposure. In 1987, NCRP Report 87 estimated that the total per capita radiation dose in the United States from natural and man-made sources is about 360 mrem per year (1); 300 mrem is from natural sources and 60 mrem is from man-made sources. The contribution from diagnostic radiology studies is 39 mrem and from nuclear medicine studies is 14 mrem. Since 1980, the per capita contribution from radiology and nuclear medicine studies has probably doubled. This increase is due to the increased use of certain diagnostic tests that involve exposure such as cardiac catheterization to detect coronary artery disease and due to the introduction of new technology such as spiral computed tomography (2–5). Doses from some interventional studies can reach levels where deterministic effects are seen (6).

Because the diagnostic use of radiation is the largest single man-made source of radiation exposure, some researchers have tried to estimate the potential harmful effects of these exposures. Such studies are plagued with numerous uncertainties including uncertainties in dose and uncertainties in dose–response relationships.

#### Sources of Uncertainty

The uncertainties in dose include variability due to technical factors and variability in how dose is defined (7). Important technical factors include the exposure rate, the number of films taken, the amount of fluoroscopy time, and the portion of the body exposed. Furthermore, dose often varies considerably with depth. Skin dose may be very different from bone marrow dose. Some have suggested that the doses to patients

be recorded, but whether any simply recorded dose (presumably skin dose) can be converted to a meaningful risk is debatable.

Most epidemiologists would like to have organ doses to convert dose to risk. Converting inhomogeneous partial-body doses into organ doses is problematic. Furthermore, many obstacles, in addition to the uncertainty in dose, hinder choosing the correct dose–response model. Organ-specific cancer risk coefficients are more uncertain than are generic risk coefficients. For radiation protection purposes, a simple linear, no-threshold dose–response model is generally assumed. It is unlikely that this simple model accurately predicts the interactions that occur in a complex biological system, and many scientists believe that this dose–response model overestimates the harmful effects of radiation, particularly at low doses. Important modifiers for medical exposures include the age and life expectancy of the exposed population. Finally, the radiation exposure results in not only potentially harmful effects but also potentially beneficial effects. Optimizing the ratio of potential benefits and risks will continue to be a challenge.

#### Conclusions

Epidemiologists who attempt to directly determine the dose–response relationship between diagnostic radiation exposure and adverse outcomes face formidable obstacles. Despite the large collective dose, it is difficult to imagine a study design that would defensibly account for the large uncertainties and confounding variables. Only in rare instances involving particularly vulnerable populations have epidemiological studies of diagnostic radiographic studies yielded results that have been accepted as valid.

The difficulty that epidemiologists have in measuring the effect from the use of diagnostic medical imaging should not be used as an excuse for having a cavalier attitude about radiation exposure in medicine. Physicians using equipment that exposes patients to radiation are often poorly trained in even basic radiation protection concepts (8). Real-time measurement of radiation exposure during interventional procedures would provide useful feedback (9). The ALARA (as low as reasonably achievable) concept should be discussed and promoted. Unfortunately, there is considerable disagreement about what is “reasonable”. This is particularly true in a medical setting when there are tangible benefits (as well as risks) associated with medical exposures.

#### References

1. NCRP, *Ionizing Radiation Exposure of the Population of the United States*. Report No. 93, National Council of Radiation Protection and Measurements, Bethesda, MD, 1987.
2. F. J. Overbeek, E. K. Pauwels, J. L. Bloem, J. A. Camps, J. Geleijns and J. J. Broerse, Somatic effects in nuclear medicine and radiology. *Appl. Radiat. Isot.* **50**, 63–72 (1999).
3. A. Perris, C. Hourdakis and A. Manetou, Examination frequencies and patient doses from computed tomography examinations in the area of Athens, Greece. *Health Phys.* **77**, 192–195 (1999).
4. D. J. Roebuck, Risk and benefit in paediatric radiology. *Pediatr. Radiol.* **29**, 637–640 (1999).
5. D. W. Ware, W. Huda and P. J. Mergo, Radiation effective doses to patients undergoing abdominal CT examinations. *Radiology* **210**, 645–650 (1999).
6. L. K. Wagner, Severe skin reactions from interventional fluoroscopy: Case report review of the literature. *Radiology* **213**, 773–776 (1999).
7. R. A. Parry, S. A. Glaze and B. R. Archer, The AAPM/RSNA physics tutorial for residents. Typical patient doses in diagnostic radiology. *Radiographics* **19**, 1289–1302 (1999).
8. L. Brateman, Radiation safety considerations for diagnostic radiology personnel. *Radiographics* **19**, 1037–1055 (1999).
9. J. T. Cusma, M. R. Bell and M. A. Wondrow, Real-time measurement of radiation exposure to patients during coronary angiography and percutaneous interventional procedures. *J. Am. Coll. Cardiol.* **33**, 427–435 (1999).

## Variations in Dose from Diagnostic Radiographic Examinations in Los Angeles County

Wendy Mack

University of Southern California, Department of Preventive Medicine,  
Los Angeles, California

(with Susan Preston-Martin, Duncan Thomas and Zhonghuan Ma,  
University of Southern California)

Radiation exposures from medical diagnostic examinations are a significant source of X-ray exposures to the U.S. and other populations (1, 2). To assess diagnostic X-ray exposures as a possible etiological agent in acute myelogenous leukemia (AML), we conducted a case-control study among residents of Los Angeles County. From each subject, we obtained a detailed history of diagnostic radiographic examinations, including the type and site of examination, the examination date, and the facility at which the examination was performed. We then attempted to obtain medical records for each reported examination.

Los Angeles County Radiation Management (LACRM) routinely monitors all X-ray machines used in the county. LACRM measures and records entrance skin exposure (ESE) and other examination-specific data for the three most common examinations conducted on any particular machine. These machine inspection records were obtained for each medical facility from which any subject-reported X-ray examination was verified with medical records. For our case-control study, the exposure period of interest was 10 years prior to diagnosis. Therefore, LACRM data were obtained and abstracted for the calendar period of 1978-1994. Although we could obtain these data by facility, we cannot link any subject-reported X-ray examination to a specific machine.

The ultimate goal in obtaining the LACRM data is to obtain a subject estimate of bone marrow dose. In this report, we use these data to (1) describe variations among machines and facilities in ESE for three common diagnostic examination sites: chest, abdomen and spine (including thoracic and lumbar spine); and (2) evaluate factors which might contribute to this variability. A total of 5856 records with ESE recorded were abstracted from LACRM. Of these, 1395 (24%) represented chest X rays, 897 (15%) represented abdominal X rays, and 639 (11%) represented X rays of the lumbar or thoracic spine. While the median ESE is consistent with guidance-level exposures reported for these examinations (3), there is a tremendous range of ESE for each examination.

In regression models, we evaluated variations in ESE (log-transformed) according to calendar year, facility type (teaching hospital, HMO, private acute care hospital, radiology group, private physician), and machine type (fixed or mobile) and manufacturer (GE, Siemens, Picker, and other). All three examinations showed a highly significant trend of decreasing ESE by calendar year. The parameter estimates for year of inspection did not vary significantly by examination type [over all three examinations,  $\beta = -0.033$  (0.004) per year]. Relative to acute care hospitals, teaching hospitals (chest and spine) and HMOs (abdomen and spine) used on average higher ESE. Relative to a mobile X-ray machine, fixed machines were associated with higher ESE for chest and abdominal examinations. Finally, there was some variation by machine manufacturer, with the Siemens machine associated with higher ESE (relative to GE) for chest and spinal examinations. Notably, the LACRM database does not include data on the year of manufacture of any specific machine. The associated model  $R^2$  and root mean squared (r.m.s.) error for each examination were: chest,  $R^2 = 13.5\%$ , r.m.s. error = 0.734; abdomen,  $R^2 = 10\%$ , r.m.s. error = 0.637; spine,  $R^2 = 8\%$ , r.m.s. error = 0.633. Thus there remains substantial variation within each examination that is not explained by machine and other health delivery factors. Remaining sources of variation may include (but are certainly not limited to) factors not measured in this study, including age of the X-ray machine, specific radiographic protocols used by a facility or technician, and level of individual technical expertise (2).

Implications of these data for modeling organ dose in our case-control

study (and other epidemiological studies assessing exposures to diagnostic radiography) are several-fold:

1. Estimates of ESE and organ dose that assign a mean value per subject/examination are likely upwardly biased.
2. The objective of such an exercise is to arrive at a probabilistic model for a subject/examination organ dose, which will incorporate both the expected dose and the residual variation around that expectation.
3. Estimation of total organ dose must also incorporate some estimate of the number of exposures. External data for the number of exposures are also available, and the numbers will also vary by many of the factors we have evaluated.

We have not evaluated doses for fluoroscopic examinations in this exercise. Such an effort would require estimates of exposure time.

### References

1. D. W. Johnson and W. A. Goetz, Patient exposure trends in medical and dental radiography. *Health Phys.* **50**, 107-116 (1986).
2. J. R. Williams and M. K. Catling, An investigation of X-ray equipment factors influencing patient dose in radiography. *Br. J. Radiol.* **71**, 1192-1198 (1998).
3. R. A. Parry, S. A. Glaze and B. R. Archer, The AAPM/RSNA physics tutorial for residents. Typical patient radiation doses in diagnostic radiology. *Radiographics* **19**, 1289-1302 (1999).

## Tinea Capitis: Uncertainties in Radiation Dose Estimates

D. Followill

Department of Radiation Physics, The University of Texas M. D.  
Anderson Cancer Center, Houston, Texas

(with M. Stovall, The University of Texas M. D. Anderson Cancer  
Center)

In the 1940s and 1950s, approximately 11,000 children in Israel received radiation therapy for tinea capitis. The purpose of the treatments was to deliver a reasonably uniform dose totaling approximately 7 Gy to the scalp to produce epilation (1, 2).

The radiation therapy consisted of five fields to the scalp (anterior, posterior, right and left laterals, and vertical), with lead shielding over the face and neck. The patients wore a cap that positioned the fields but were not immobilized during treatment. The beams were superficial X rays (70-100 kVp, half-value layer of approximately 1.0 mm aluminum).

Several investigators have assessed the late effects of the radiation therapy, including the incidence of thyroid tumors (3, 4). The latter study (4) is reviewed here to ascertain the sources and magnitude of uncertainties in the dosimetry and what impact these may have on the risk estimates for radiation-induced thyroid tumors.

The principal sources of uncertainties in the dosimetry are related to patient treatment; these include patient movement during treatment as well as errors in calibration of machine output, patient set-up (including target-to-skin distance), machine-on time, constancy of machine output during treatment, and documentation of treatment parameters. Of lesser magnitude are the uncertainties inherent in the methods of estimating dose to the thyroid, such as phantom measurements and associated calculations.

### References

1. H. G. Adamson, A simplified method of X-ray application for the cure of ringworm of the scalp; Kienbock's method. *Lancet* **1**, 1378-1380 (1909).
2. R. J. Schulz and R. E. Albert, Follow-up study of patients treated by X-ray epilation for tinea capitis. III. Dose to organs of the head from

the X-ray treatment of tinea capitis. *Arch. Environ. Health* **17**, 935–950 (1968).

3. B. Modan, E. Ron and A. Werner, Thyroid cancer following scalp irradiation. *Radiology* **123**, 741–744 (1977).
4. E. Ron, B. Modan, D. Preston, E. Alfandary, M. Stovall and J. D. Boice, Jr., Thyroid neoplasia following low-dose radiation in childhood. *Radiat. Res.* **120**, 516–531 (1989).

### Thyroid Cancer after Scalp Irradiation: A Reanalysis Accounting for Uncertainty in Dosimetry

Raymond J. Carroll

Texas A&M University, College Station, Texas

(with Daniel W. Schafer, Oregon State University; Jay H. Lubin, National Cancer Institute; Elaine Ron, National Cancer Institute; and Marilyn Stovall, The University of Texas M. D. Anderson Cancer Center)

In the 1940s and 1950s, approximately 11,000 children in Israel received radiation therapy for tinea capitis (ringworm of the scalp). The purpose of the treatments was to deliver a reasonably uniform dose of approximately 7 Gy to the scalp to produce epilation (1, 2). The radiation therapy consisted of five fields to the scalp (anterior, posterior, right and left laterals, and vertical), with lead shielding over the face and neck. The patients wore a cap that positioned the fields, but they were not immobilized during treatment. The beams were X rays, 70–100 kVp, half-value layer of approximately 1.0 mm aluminum.

Several investigators have assessed the late effects of radiation therapy with these data, including the incidence of thyroid tumors (3–5). The thyroid gland in the young is highly sensitive to the carcinogenic effects of ionizing radiation. Ron *et al.* (4) note that the Israel study “is one of the few human studies reporting a significant risk of cancers at doses on the order of 10 cGy”.

The major issue for this paper involves the dosimetry in converting a recorded course of radiation therapy into a dose to the thyroid. In their analysis of the tinea capitis data, Ron *et al.* (4) used the results of anthropomorphic phantom studies (2, 3, 6) to construct a dose to the thyroid from the child’s age at first irradiation, filtration of the X-ray machine, prescribed radiation beam exposure (in roentgens), and number of treatments.

At the time of the analysis reported by Ron *et al.* (3), the potential biases due to dose imprecision in the estimates of dose in relative risk regression were not as widely appreciated as they are today. More recent articles (7–13) have heightened the awareness of the problem and have provided solutions in various situations. The workshop *Uncertainties in Radiation Dosimetry and Their Impact on Dose-Response Analysis* (14) was particularly influential in motivating a re-examination of the tinea capitis data.

Our purpose is to reconsider the tinea capitis study to see whether the thyroid radiation dose uncertainties have an effect on the reported dose-response relationship and on the modifying effects of age at exposure. We will also provide a reanalysis that accounts for uncertainties. A major component of this work is the formal incorporation of “external prediction data” into the analysis. By this we mean something like the standard idea of “external validation data” (15, Chapter 1) in which dose and estimated dose are available for an external data set, the difference in the tinea capitis data being that instead we observe only estimated dose and predictor variables for dose. The use of an externally estimated prediction equation leads to a multiplicative Berkson-type model, but with a classical measurement error component due to the estimation of parameters in the prediction equation. Two additional difficulties complicate the analysis: the dose predictor variables are missing for many patients, and the use of the external data set, which is based on phantoms (simulated human bodies exposed in the same way as actual patients), misses some of the sources of dose uncertainty in live humans. Some speculation is there-

fore necessarily required, and sensitivity analysis is used to study the ramifications of this speculation.

The uncertainties in thyroid radiation dose in the tinea capitis study are due to a variety of factors, particularly the following.

#### Missing data for predicting dose:

1. Age at second and subsequent exposures is missing for the 9% of the subjects with more than one exposure.
2. The machines used, their filtration, and the prescribed beam exposures to the scalp are not known for many patients.

#### Berkson-type errors:

1. Within-individual effects, reflecting the different thyroid doses that would occur if a child were hypothetically irradiated twice under ideal conditions.
2. Between-individual effects, reflecting the different thyroid doses that would occur for different children of identical ages (rounded) under ideal machine conditions, due to differences in head size and shape.
3. Random errors due to differences between prescribed and actual skin exposure.

#### Classical measurement errors:

1. The error model includes two parameters that control the relationship between added filtration and dose, and these parameters are unknown and must be estimated. When they are estimated from phantom studies, which act as a type of classical measurement error, although shared among all individuals.
2. The variances of the Berkson-type errors are unknown and must be estimated. In principle, the error in this estimation is of the same classical type as the error in estimating the parameters described above. Because these error variances are estimated with little precision, we have chosen to perform a sensitivity analysis for them, finding little sensitivity.

We have developed models that account for these uncertainties, and methods of estimation and inference for them. In particular, we developed the idea of a calibrated likelihood, which is similar to the standard regression calibration or substitution algorithm, but which uses the likelihood contribution about uncertainty parameters from external sources. Statistical estimation and inference were based on the techniques of Breslow *et al.* (16).

Our results were striking in finding that accounting for uncertainty had little effect. Parameter estimates, standard errors and inferences were all little affected by accounting for the uncertainty. We believe this is because the relative risk model is linear in dose, and because much of the uncertainty is of Berkson-type.

#### References

1. H. G. A. Adamson, Simplified method of X-ray application for the cure of ringworm of the scalp: Kienbock’s method. *Lancet* **1**, 1378–1380 (1909).
2. R. J. Schulz and R. E. Albert, Follow-up study of patients treated by x-ray epilation for tinea capitis III: Dose to organs of the head from the x-ray treatment of tinea capitis. *Arch. Environ. Health* **17**, 935–950 (1968).
3. B. Modan, E. Ron and A. Werner, Thyroid cancer following scalp irradiation. *Radiology* **123**, 741–744 (1977).
4. E. Ron, B. Modan, D. Preston, E. Alfandary, M. Stovall and J. D. Boice, Jr., Thyroid neoplasia following low-dose radiation in childhood. *Radiat. Res.* **120**, 516–531 (1989).
5. E. Ron, J. H. Lubin, R. E. Shore, K. Mabuchi, B. Modan, L. M. Pottern, A. B. Schneider, M. A. Tucker and J. D. Boice, Jr., Thyroid cancer after exposure to external radiation: A pooled analysis of seven studies. *Radiat. Res.* **141**, 259–277 (1995).

6. W. Lee and H. D. Youmans, *Doses to the Central Nervous System of Children Resulting from X-ray Therapy for Tinea Capitis*. Technical Report BRH/DBE 70-74, U.S. Department of Health, Education and Welfare, Public Health Service, Washington, DC, 1970.
7. M. S. Pepe, S. G. Self and R. L. Prentice, Further results in covariate measurement errors in cohort studies with time to response data. *Stat. Med.* **8**, 1167–1178 (1989).
8. D. A. Pierce, D. O. Stram and M. Vaeth, Allowing for random errors in radiation dose estimates for the atomic bomb survivors. *Radiat. Res.* **123**, 275–284 (1990).
9. D. A. Pierce, D. O. Stram, M. Vaeth and D. Schafer, Some insights into the errors in variables problem provided by consideration of radiation dose–response analyses for the A-bomb survivors. *J. Am. Stat. Assoc.* **87**, 351–359 (1992).
10. T. Nakamura, Proportional hazards models with covariates subject to measurement error. *Biometrics* **48**, 829–838 (1992).
11. M. D. Hughes, Regression dilution in the proportional hazards model. *Biometrics* **49**, 1056–1066 (1993).
12. D. Thomas, D. Stram and J. Dwyer, Exposure measurement error: influence on exposure–disease relationships and methods of correction. *Annu. Rev. Pub. Health* **14**, 69–93 (1993).
13. J. H. Lubin, J. D. Boice, Jr. and J. M. Samet, Errors in exposure assessment, statistical power and the interpretation of residential radon studies. *Radiat. Res.* **144**, 329–341 (1995).
14. E. Ron and F. O. Hoffman, *Uncertainties in Radiation Dosimetry and Their Impact on Dose Response Analysis*. National Cancer Institute Press, 1999.
15. R. J. Carroll, D. Ruppert and L. A. Stefanski, *Measurement Error in Nonlinear Models*. Chapman and Hall, London, 1995.
16. N. E. Breslow, J. H. Lubin, P. Marek and B. Langholz, Multiplicative models and cohort analysis. *J. Am. Stat. Assoc.* **78**, 1–12 (1983).

### Uncertainty of Response to Ionizing Radiation due to Genotype: Potential Role for Variation in DNA Repair Genes

Harvey W. Mohrenweiser

*Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California*

(with Irene M. Jones, Lawrence Livermore National Laboratory)

The risk of cancer after exposures to environmental agents and lifestyle factors is influenced by the genetic constitution of the individual. The role of genetics in the individual risk of cancer is most apparent for the known cancer genes, e.g. variants of the breast cancer genes *BRCA1* and *BRCA2* and variants of the nucleotide excision repair genes associated with xeroderma pigmentosum. Although the individuals with these highly penetrant variant alleles are at very high risk for cancer, these variants account for less than 5% of the cases in the population. Accumulating evidence suggests that most cases of cancer are associated with low to moderate levels of exposure in genetically susceptible individuals; that is, most cancer results from the interaction of common, low-penetrant genetic variants with environmental exposure or lifestyle. These polymorphisms in susceptibility genes are associated with small elevations in risk in exposed individuals but have the potential to have a significant impact on the population incidence of cancer because of the large number of individuals at risk.

Although genetic susceptibility to cancer has been widely studied in relation to chemical carcinogenesis, only limited data are available on the potential for genetic variation to be a population risk factor for the consequences of radiation exposure. DNA repair proteins are involved in the removal or repair of DNA damage and thus are critical in protecting cells from the consequences of exposure to carcinogenic agents, including radiation. The DNA damage recognition and cell cycle checkpoint genes

also have roles in the repair of damaged DNA and in cancer susceptibility. The regulation of the timing of DNA replication by cell cycle checkpoint genes has an impact on the extent of DNA repair and the consequences of reduced repair. It is the level of unrepaired damage at the time of cell division that is critical for determining the consequences of DNA damage. Genetic variation that reduces the function of these pathways, especially variant alleles present in a significant portion of the population, could affect the population cancer incidence after exposures to low to moderate levels of cancer-causing agents.

#### *Variation in Response to Radiation*

A genetic basis for variation in response to radiation has been established in both humans and mice. These studies suggest an important role for DNA repair. An example of genetic variation in a cancer-predisposing gene is *ATM*, where several studies have reported that *ATM* heterozygotes are at increased risk for breast cancer (1). It has been suggested that the increased breast cancer risk in *ATM* heterozygotes is expressed after radiation exposure. However, other studies have reported that *ATM* heterozygosity is not a risk factor for early-onset breast cancer (2). Cells deficient in *ATM* are hypersensitive to ionizing radiation and exhibit abnormal DNA repair, consistent with its role in regulation of the cell cycle (3). Several human genetic diseases, e.g. Nijmegen breakage syndrome, are associated with increased sensitivity to ionizing radiation and cancer proneness. Bloom's syndrome and Fanconi's anemia are examples of other diseases exhibiting sensitivity to ionizing radiation and marginally increased cancer risk. Studies of inbred strains of mice, where differences in both tumor incidence and lethality after radiation exposure are noted, provide the most definitive support for genetic variation in susceptibility to radiation-induced cancer and sickness. Storer *et al.* (4) have summarized a series of studies showing that the incidence of several tumors differed among different mouse strains after radiation exposure. Ullrich and colleagues (5) have related the high sensitivity of BALB/c mice to radiation-induced mammary tumors to the high level of radiation-induced chromosome instability in this strain and sequence variation in the gene encoding DNA-PK. The difference in ability to repair radiation-induced double-strand breaks noted between the BALB/c (sensitive) and C57BL6 (resistant) mouse strains is consistent with a role for DNA-PK and DNA repair in the differential response to radiation exposure. Thus a precedent for variation in individual susceptibility to ionizing radiation-induced cancer and a role DNA repair has been established. Chakraborty and Sankaranarayanan (6) have modeled the relationship of genetic variation, radiation dose and cancer risk and identified 30 genes as potential "cancer-predisposing genes".

#### *DNA Repair Capacity as an Indicator of Cancer Susceptibility*

Further support for a role of DNA repair in cancer susceptibility comes from a series of lymphocyte-based studies reporting that the repair capacity for several classes of DNA damage is a polymorphic trait. Approximately 20% of healthy individuals exhibit a repair capacity that is 65–80% that of the overall population mean. The repair capacities for damage induced by bleomycin,  $\gamma$  radiation and BPDE are independent traits and the heritability of the repair capacity phenotypes is 0.63–0.80, characteristics expected of genetic traits (Wu *et al.*, unpublished results). It has been observed in a large number of case-control studies that individuals with reduced repair capacity have a higher probability of being in the cancer cohort than in the control cohort. It should be noted that these repair capacity assays sum the activity and functionality of the total pathway but do not provide direct evidence for reduced function at any specific step or gene.

#### *Molecular Basis for Variation in DNA Repair Capacity*

Studies in mammalian cells have identified more than 40 genes with roles in the processing of radiation-induced DNA damage. Studies of these cells confirm that the loss of function of DNA repair, DNA damage recognition, and cell cycle checkpoint genes is associated with impaired

ability to repair DNA damage induced by ionizing radiation and the consequent reduced cell survival. Identification and sequencing of these genes provides the information necessary to screen for genetic variation.

To provide the reagents necessary for molecular epidemiology studies that directly address the hypothesis that polymorphic variation in DNA repair and repair-related genes is associated with cancer risk, we have initiated a program to resequence the exons and adjacent regions in the introns of these genes. Thus far, 19 DNA repair or repair-related genes have been screened for variation by resequencing of DNA from 36–92 individuals (7). A total of 280 single nucleotide polymorphisms (SNPs) have been identified, including 48 different amino acid substitution variants. An average of more than two amino acid substitutions per gene were detected with an average variant frequency of ~8% in this small set of individuals. Several DNA repair gene variants exist at sufficient frequency that homozygous variant individuals, individuals with two different variant alleles at one locus, or individuals with variant alleles for several genes in the same pathway have been observed. Approximately 50% of the individuals screened have variant subunits of two proteins that are components of multimeric complexes or genes comprising sequential steps of a pathway. Approximately 50% of the amino acid substitutions are non-conservative amino acid replacements at amino acid residues that are identical in humans and mice. This conservation suggests that the variation is occurring at residues that may be significant for normal protein function and that the variant proteins may exhibit reduced function. Biochemical characterization of variants of the major apurinic endonuclease indicated that four of seven variants had significantly reduced catalytic function. Nine of the genes screened for variation thus far have roles in base excision repair, and four have roles in repair of double-strand breaks or damage recognition, pathways important for repair of damage induced by ionizing radiation. Future studies will require that all of the genes with roles in relevant pathways be screened for variation so that the genotyping studies will emulate the repair capacity phenotyping studies in monitoring the function of the entire pathway.

#### Risk Associated with Repair Gene Variants

Epidemiology studies are beginning to address questions of the health relevance of variation in DNA repair genes. The variants identified in the DNA repair gene resequencing are in the public domain and are reagents for both biochemical characterization and molecular epidemiology studies. Several collaborators have used this information to develop genotyping assays for studies of cancer cohorts and controls with a goal of identifying associations of variants with increased cancer risk or phenotypes presumed to be indicative of cancer risk. Examples include associations of variation in XPD with risk of basal cell carcinoma (8) and XRCC1 with smoking-related cancers (9). XRCC1 variation has been associated with increased levels of aflatoxin adducts and an increased frequency of GPA mutations and sister chromatid exchanges in smokers (10, 11).

#### Conclusion

A range of strategies, including biochemistry, cell biology and epidemiology, will be required to define the functional significance and health consequences of genetic variants in these DNA repair and repair-related genes. Understanding the impact of inherited genetic variation in genes that repair damaged DNA on individual susceptibility to cancer will require studies in large cohorts to have sufficient sample size to support gene–gene interaction studies. It will also require that the exposures be well characterized so that the gene–exposure (dose) interaction can be characterized. Very large studies will be critical for predicting the human health consequences of low-level exposure to cancer-causing agents, including ionizing radiation.

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#### References

1. P. Athma, R. Rappaport and M. Swift, Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. *Cancer Genet. Cytogenet.* **92**, 130–134 (1996).
2. M. G. FitzGerald, J. M. Bean, S. R. Hagde, H. Unsal, D. J. MacDonald, D. P. Harkin, D. M. Finkelstein, K. J. Isselbacher and D. A. Haber, Heterozygous ATM mutations do not contribute to early onset of breast cancer. *Nat. Genet.* **15**, 307–310 (1997).
3. M. F. Lavin and Y. Shiloh, The genetic defect in ataxia-telangiectasia. *Annu. Rev.* **15**, 177–202 (1997).
4. J. B. Storer, T. J. Mitchell and R. J. M. Fry, Extrapolation of the relative risk of radiogenic neoplasms across mouse strains and to man. *Radiat. Res.* **114**, 331–353 (1988).
5. B. Ponnaiya, M. N. Cornforth and R. L. Ullrich, Radiation-induced chromosomal instability in BALB/c and C57BL/6 mice: The difference is as clear as black and white. *Radiat. Res.* **147**, 121–125 (1997).
6. R. Chakraborty, M. P. Little and K. Sankaranarayanan, Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. III. Effects of incomplete penetrance and dose-dependent radiosensitivity on cancer risks in populations. *Radiat. Res.* **147**, 309–320 (1997).
7. H. Mohrenweiser and I. M. Jones, Variation in DNA repair is a factor in cancer susceptibility: A paradigm for the promises and perils of individual and population risk estimation. *Mutat. Res.* **400**, 15–24 (1998).
8. M. Dybdahl, U. Vogel, G. Frentz, H. Wallin and B. A. Nexø, Polymorphisms in the DNA repair gene XPD: Correlations with risk and age at onset of basal cell carcinoma. *Cancer Epidemiol. Biomarkers Prev.* **8**, 77–81 (1999).
9. E. M. Sturgis, E. J. Castillo, L. Li, R. Zheng, S. A. Eicher, G. L. Clayman, S. Strom, M. R. Spitz and Q. Wei, Polymorphisms of DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis* **21**, 965–971 (2000).
10. E. J. Duell, J. K. Wiencke, T. J. Cheng, A. Varkonyi, Z. F. Zuo, T. D. Ashok, E. J. Mark, J. C. Wain, D. C. Christiani and K. T. Kelsey, Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis* **21**, 965–971 (2000).
11. R. M. Lunn, R. G. Langlois, L. L. Hsieh, C. L. Thompson and D. A. Bell, XRCC1 polymorphisms: Effects on aflatoxin B<sub>1</sub>-DNA adducts and glycophorin A variant frequency. *Cancer Res.* **59**, 2557–2561 (1999).

#### DISCUSSION: Uncertainties in Exposure and Response

Susan Preston-Martin

Keck School of Medicine, University of Southern California, Los Angeles, California

Henry Royal has pointed out that medical exposures are by far the largest man-made source of population exposure to ionizing radiation and that diagnostic exposures are the predominant contributor to this. David Followill and Ray Carroll have discussed the many sources of uncertainty in estimates of the radiation dose to those in the Israeli tinea capitis cohort, for whom considerable data on radiation parameters were recorded. The uncertainties involved in estimating doses from past radiographic procedures are far more daunting. For diagnostic procedures, far less is recorded. No information is available in medical charts on critical dose-related details such as what machine setting was used or the length of time of fluoroscopic examinations. Wendy Mack presented comparison dosimetry data on X-ray machines currently in use in Los Angeles County that show wide variation in the radiation dose to the patient during several specific types of radiographic examinations. But no information is available in medical charts in the county on which machine was used for a recorded radiographic examination. Our County Hospital, the pri-

many teaching hospital for USC Medical School, has more than a hundred X-ray machines in current use.

This dose uncertainty is becoming an increasingly formidable obstacle in studies of radiographic exposures. In a dental X-ray validation study we did 15–20 years ago as part of a study of salivary gland tumors, we were able to estimate doses. Even when the radiation parameters were not recorded in the dental chart and the dentist did not remember the machine settings or what speed film he had used in a given calendar year, we were able to make assumptions that allowed us to estimate doses. For example, Kodak's sales records allowed us to know the year most dentists switched to a new faster-speed film. For this study we asked patients with salivary gland tumors and their controls about all the dentists they had gone to and how often each had X-rayed their teeth. When we compared interview information to that from dental charts, we learned that recall of dental X rays appeared to be unbiased. We also learned that 85% of radiation exposure to the salivary glands came from dental X rays. Furthermore, we found a clear, statistically significant dose–response relationship between cumulative exposure of the salivary glands from dental X rays and risk of salivary gland cancer.

The situation today is vastly different when trying to estimate doses from an increasingly broad range of medical radiographic procedures. We are currently trying to finish the analysis of a subtype-specific study of adult-onset acute myelogenous leukemia (AML). We have done a thorough literature search, searched the Web, and consulted numerous recommended experts and have estimated doses for more than 200 different types of radiographic examinations which the 850 subjects in our study had in the 10 years before diagnosis. Nonetheless, we have no idea what dose to assign to dozens of types of examinations. This obstacle is becoming more formidable with the rapid proliferation of imaging procedures. Investigators need to be able to estimate dose from all these types of examinations in future studies to estimate sources of population exposure and to evaluate possible dose–response relationships. The studies of the gene–environment interactions Harvey Mohrenweiser discussed will not be possible unless we also consider radiographic exposures. As he stressed, most cancer is associated with low to moderate levels of exposure in genetically susceptible individuals.

Therefore, I am making an appeal to all of you here to point us to appropriate help in tracking down dose estimates for three groups of radiographic examinations. The first includes diagnostic imaging procedures other than nuclear scans where someone has given us a guess as to what the dose might be, but we have been unable to track down a defensible dose estimate. The second includes the “guesstimates” we have been given for various nuclear medicine scans. The third includes nuclear medicine scans for which we have no dose estimates whatsoever. If any of you can help us with pinning down doses for any of these examinations, please let me know.

In closing, I want to say how glad I am that the ASA agreed to include a session on medical exposure at this meeting. Indeed, a session on issues relating to medical exposures would be appropriate to have at every ASA radiation meeting given that this is the number one source of population exposure from man-made sources of ionizing radiation.

### III. THE EVALUATION OF DISEASE RISKS

Chair: Amy Kronenberg

*Lawrence Berkeley National Laboratory, Berkeley, California*

#### **Predisposition, Susceptibility and DNA Repair in Radiation-Induced Skin Cancer**

Kenneth H. Kraemer

*Basic Research Laboratory, National Cancer Institute, Bethesda, Maryland*

People with the rare genetic disorder xeroderma pigmentosum (XP) have marked sensitivity to sunlight, multiple pigmented lesions, and skin

cancer at an early age (1–3). XP patients have an approximately 1000-fold increased risk of developing cutaneous basal cell carcinoma, squamous cell carcinoma, and melanoma (3). The average age of first skin cancer in XP patients is less than 10 years—a 50-year reduction in comparison to the U.S. general population (4). Cells from XP patients are hypersensitive to killing by ultraviolet (UV) radiation and are hypermutable after exposure to UV radiation. These abnormalities are caused by defective DNA repair.

There are seven DNA repair genes (*XPA* to *XPG*) involved in nucleotide excision repair and another gene (*XPV*) involved in bypass of UV photoproducts (1). Defects in these genes are associated with different clinical forms of XP. They result in large (50–100%) reductions in excision repair.

Aging in the general population is associated with a progressive small decline (about 0.6% per year) in DNA repair (5). The mechanism of this decline has not been discovered. People with skin cancer may have a more rapid decline in DNA repair with age.

Mutations in DNA repair genes that do not appear to greatly alter their function are now being identified. If their frequency is at least 1% in the general population, they are termed “polymorphisms”. Some of these polymorphisms in coding sequences result in changes in amino acids in the affected proteins, and others may change the tRNA recognition sequences while preserving the amino acids. Changes in introns may alter gene function in presently unknown ways. Polymorphisms in DNA repair genes may play a role in cancer susceptibility and are currently being intensively investigated (6).

#### *References*

1. D. Bootsma, K. H. Kraemer, J. E. Cleaver and J. H. J. Hoeijmakers, Nucleotide excision repair syndromes: Xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. In *The Genetic Basis of Human Cancer* (B. Vogelstein and K. W. Kinzler, Eds.), pp. 245–274. McGraw-Hill, New York, 1998.
2. K. H. Kraemer, M. M. Lee and J. Scotto, Xeroderma pigmentosum: Cutaneous, ocular and neurologic abnormalities in 830 published cases. *Arch. Dermatol.* **123**, 241–250 (1987).
3. K. H. Kraemer, M-M. Lee, A. D. Andrews and W. C. Lambert, The role of sunlight and DNA repair in melanoma and non-melanoma skin cancer: The xeroderma pigmentosum paradigm. *Arch. Dermatol.* **130**, 1018–1021 (1994).
4. J. Scotto, T. R. Fears, J. H. Kraemer and J. F. Fraumeni, Jr., Non-melanoma skin cancer. In *Cancer Epidemiology and Prevention*, 2nd ed. (D. Schottenfeld and J. F. Fraumeni, Jr., Eds.), pp. 1313–1330. Oxford University Press, New York, 1996.
5. S-I. Moriwaki, S. Ray, R. E. Tarone, K. H. Kraemer and L. Grossman, The effect of donor age on the processing of UV-damaged DNA by cultured human cells: Reduced DNA repair capacity and increased DNA mutability. *Mutat. Res. DNA Repair* **364**, 117–123 (1996).
6. S. G. Khan, E. J. Metter, R. E. Tarone, V. A. Bohr, L. Grossman, M. Hedayati, S. Bale, S. Emmert and K. H. Kraemer, A new xeroderma pigmentosum group C poly-AT intron insertion/deletion polymorphism. *Carcinogenesis* **21**, 1821–1825 (2000).

#### **Cancer Predisposition, Radiosensitivity and the Risk of Radiation-Induced Cancers: Biological Aspects and Computational Modeling**

K. Sankaranarayanan

*Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, The Netherlands*

It has long been known that among human Mendelian diseases (i.e. hereditary diseases due to mutations in single genes), there is a subset in which a cancer of one type or another is the sole or frequent phenotypic

manifestation of the mutant gene. Individuals with such mutant genes are said to be cancer-predisposed, cancer-prone or cancer-susceptible. Mulvihill's 1999 compilation (1) shows that 635 (6.2%) entries in McKusick's 1998 compendium (2) represent genes and/or disorders that predispose to or are associated with neoplasia. Interest in the question of whether individuals with such disorders or their cells would be radiosensitive was catalyzed by two important discoveries in the late 1960s: First, in 1968, Cleaver demonstrated that in patients with xeroderma pigmentosum, an autosomal recessive disorder, defective DNA repair is the biochemical cause for cellular UV-radiation hypersensitivity, which in turn leads to solar radiation-induced skin cancers. Around the same time, other investigators found that patients with ataxia telangiectasia, another autosomal recessive disorder reacted catastrophically to conventional X-ray therapy.

Research carried out during the last several years lends support to the view that some of the genes underlying cancer predisposition may also make their carriers more sensitive to ionizing radiation-induced cancers. If this were true, (a) individuals who carry the radiosensitive cancer-predisposing gene(s) may be at a higher risk for induced cancers than those who do not have them, (b) the risk of radiation-induced cancers in a population in which these radiosensitive subgroups exist may be higher than in a population which does not have these subgroups, and (c) the risk of radiation-induced cancers to relatives of cancer-predisposed individuals will be higher (because of the enrichment of cancer-predisposing mutations in them) than to unrelated individuals.

To study this problem, a Mendelian autosomal one-locus, two-allele model was developed. The model assumes that one of the alleles is mutant and that the genotypes carrying the mutant allele are cancer-predisposed and are also more sensitive to radiation-induced cancer. The model allows for the dose dependence of radiosensitivity differentials among genotypes and incomplete penetrance (i.e., not all individuals who carry the mutant allele express the cancer phenotype); additionally, it takes into account the possibility that not all cancers of a given type are due to mutations in the gene under study. The following specific questions were addressed: (a) If a population is heterogeneous (i.e. consisting of cancer-predisposed and non-predisposed subgroups) and if the predisposed subgroup is also more sensitive to radiation-induced cancers, how much higher will the radiation cancer risks be in such a heterogeneous population, compared to one which is homogeneous in this regard? (b) Under the same circumstances, what will the increase in radiation cancer risks be in relatives of cancer-predisposed individuals compared to that in unrelated individuals? Formal analytical predictions were made with the model, and these were illustrated using recent data on breast cancers due to the *BRCA1* gene (3-5).

The following results have been obtained: (a) When the population is heterogeneous with respect to cancer predisposition and radiosensitivity, irradiation results in a greater increase in the frequency of induced cancers; the relative risks (i.e. the ratio of cancer risks in a heterogeneous population relative to that in a homogeneous population) increases with increasing dose, but the dose dependence of relative risks diminishes at higher doses. (b) The attributable fraction (AF, the proportion of increase in risk that is due to both increased susceptibility and increased radiosensitivity) and the proportion of attributable risk due to increased radiosensitivity alone increase with increasing dose, and the dose dependence of each measurement also diminishes at higher doses. (c) When the proportion of cancers due to susceptible genotypes is small (<10%, as is likely to be the case for breast cancer in non-Ashkenazi Jewish women), the increases in the relative risk and attributable risk are marked only when there are very large increases in cancer susceptibility (>1000-fold) and radiosensitivity (>100-fold) in the susceptible group. (d) When the proportion of cancers due to the susceptible genotypes is appreciable (i.e. >10%, as may be the case for breast cancer in Ashkenazi Jewish women), there may be large increases in the relative risk and attributable risk for comparatively moderate increases in cancer susceptibility (>10-fold) and radiosensitivity (>100-fold) in the susceptible subpopulation. (e) For values of predisposition strength and radiosensitivity differential <10, even when the estimated frequency of a mutant *BRCA1* gene is 0.0047 and the proportion of breast cancers due to these mutations is 38% (as is the case

for Ashkenazi Jewish women under age 30), the increase in breast cancer risks is only marginal even for first-degree relatives. (f) For any given combination of strength of predisposition and radiosensitivity differential, incomplete penetrance dilutes the effect.

These results support the general conclusion that increases in radiation cancer risks to a heterogeneous population to be detectable epidemiologically will occur only when the mutant alleles are common and the strengths of predisposition and radiosensitivity differentials are conjointly dramatic.

#### References

1. J. J. Mulvihill, Catalog of human cancer genes. In *McKusick's Mendelian Inheritance in Man for Clinical and Research Oncologists (Onco-MIM)*. Johns Hopkins University Press, Baltimore, MD, 1999.
2. V. A. McKusick, *Mendelian Inheritance in Man*, 10th ed. Johns Hopkins University Press, Baltimore, MD, 1998.
3. R. Chakraborty, M. P. Little and K. Sankaranarayanan, Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. III. Effects of incomplete penetrance and dose-dependent radiosensitivity on cancer risks in populations. *Radiat. Res.* **147**, 309-320 (1997).
4. R. Chakraborty, M. P. Little and K. Sankaranarayanan, Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. IV. Prediction of risks in relatives of cancer-predisposed individuals. *Radiat. Res.* **149**, 493-507 (1998).
5. R. Chakraborty and K. Sankaranarayanan, Mutations in the *BRCA1* gene: Implications of inter-population differences for predicting the risk of radiation-induced breast cancers. *Genet. Res.* **72**, 191-198 (1998).

### Hemochromatosis Heterozygotes May Constitute a Radiation-Sensitive Subpopulation

R. G. Stevens

*University of Connecticut Health Center, Farmington, Connecticut*

A primary mechanism of radiation-induced DNA damage is by generation of free radicals. Chronically increased oxidative stress from elevated body iron may increase radiation sensitivity by decreasing cellular oxygen radical scavenging capability. Hemochromatosis heterozygotes have elevated body iron. Low-level radiation sensitization by iron may be particularly pertinent for risk of breast cancer. Since 10% of the population appears to be heterozygous for the hemochromatosis gene, a radiosensitizing effect would have pervasive implications.

Hemochromatosis is a genetic condition (termed *HFE* and located on chromosome 6) that leads to a morbid accumulation of body iron. Untreated hemochromatosis is physically catastrophic. In addition, more than 10% of the population is heterozygous for *HFE* mutated genes (1), and heterozygotes have moderately elevated body iron stores (2). The disease hemochromatosis is a clear example of a gene-environment interaction. *HFE*-normal people maintain an effective intestinal block to the absorption of dietary iron once they are iron-replete; non-heme iron is not absorbed, and very little heme-iron is absorbed (3). In contrast, iron in the diet, particularly heme-iron, is readily absorbed by *HFE*-mutation homozygotes well beyond an iron-replete body iron status. Heterozygotes have an intermediate phenotype in which some block is induced when iron-replete, but more iron than necessary is still absorbed. It is important to note that *HFE* is common in people of northern European ethnicity, but uncommon in other ethnic groups (1). Gordeuk *et al.* (4) reported on an iron overload genetic predisposition in parts of Africa that is not linked to *HFE* yet is very common in the population studied. Other iron-overload genes should be pursued vigorously.

Oxidative stress and free radical processes are believed to contribute to the pathogenesis of many maladies including cancer, heart disease,

arthritis and neurodegenerative disorders (5). Elevated body iron has been reported to be associated with increased cancer risk (6, 7), perhaps by generation of free radicals (8).

Breast cancer can be caused by radiation exposure (9), and a primary mechanism for radiation-induced DNA damage is generation of free radicals; this is true for low- (10) and high- (11) LET radiation. Therefore, elevated body iron may increase sensitivity to low-dose radiation-induced cancer, particularly of breast, through chronic oxidative stress leading to a compromise of oxidative defense mechanisms (12).

Zhou *et al.* (13) reported that cell-cell communication greatly facilitates the bystander effect, in which unirradiated cells suffer DNA damage when neighboring cells are irradiated. Although DMSO had no effect on the bystander effect, this does not rule out a role for free radical biology. Iliakis *et al.* (14) reported that hydrogen peroxide induces DNA double-strand breaks in Chinese hamster ovary cells at very low concentrations ( $\sim 1 \mu\text{M}$ ), much lower than required for cell killing. The authors speculate that iron-DNA complexes allow for Fenton chemistry when hydrogen peroxide is present and production of hydroxyl radicals in proximity to the DNA. Therefore, the bystander effect may be influenced by production of hydrogen peroxide in irradiated cells diffusing through gap junctions to DNA of unirradiated cells where an iron complex could rapidly damage the DNA by Fenton chemistry. High intracellular iron content would be expected to increase the bystander effect and low iron to inhibit it. Catalase would be expected to reduce or eliminate the bystander effect if hydrogen peroxide is involved.

These hypotheses can be tested by application of biomarkers of oxidative stress which offer the potential for elucidating mechanisms of biomolecular damage from many sources including endogenous biochemistries, genetic polymorphisms, occupational or environmental xenobiotic exposures, and diet. However, use of biomarkers of oxidative stress suffers from two dangers: (1) that the chosen biomarker does not actually reflect damage relevant to pathogenesis, and (2) that it is very difficult to measure accurately due to artifact in isolation. Despite these concerns, there is considerable interest in pursuing this direction for research on mechanisms of disease causation (15).

#### References

1. W. Burke, E. Thomson, M. J. Khoury, S. M. McDonnell, N. Press, P. C. Adams, J. C. Barton, E. Beutler, G. Brittenham and F. S. Collins, Hereditary hemochromatosis: gene discovery and its implications for population-based screening. *J. Am. Med. Assoc.* **280**, 172–178 (1998).
2. Z. J. Bulaj, L. M. Griffen, L. B. Jorde, C. Q. Edwards and J. P. Kushner, Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. *N. Engl. J. Med.* **335**, 1799–1805 (1996).
3. J. D. Cook, Adaptation in iron metabolism. *Am. J. Clin. Nutr.* **51**, 301–308 (1990).
4. V. Gordeuk, J. Mukiiki, S. J. Hasstedt, W. Samowitz, C. Q. Edwards, G. West, S. Ndambire, J. Emmanuel, N. Nkanza and G. Brittenham, Iron overload in Africa: Interaction between a gene and dietary iron content. *N. Engl. J. Med.* **326**, 95–100 (1992).
5. B. Halliwell and J. M. C. Gutteridge, Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.* **186**, 1–85 (1990).
6. R. G. Stevens, R. P. Beasley and B. S. Blumberg, Iron-binding proteins and risk of cancer in Taiwan. *J. Natl. Cancer Inst.* **76**, 605–610 (1986).
7. R. G. Stevens, D. Y. Jones, M. S. Micozzi and P. R. Taylor, Body iron stores and the risk of cancer. *N. Engl. J. Med.* **319**, 1047–1052 (1988).
8. S. Toyokuni, Iron-induced carcinogenesis: the role of redox cycling. *Free Radic. Biol. Med.* **20**, 553–566 (1996).
9. E. Ron, Ionizing radiation and cancer risk: Evidence from epidemiology. *Radiat. Res.* **150** (Suppl.), S30–S41 (1998).
10. J. E. Repine, O. W. Pfenninger, D. W. Talmage, E. M. Berger and D. E. Pettijohn, Dimethyl sulfoxide prevents DNA nicking mediated by ionizing radiation or iron/hydrogen peroxide-generated hydroxyl radical. *Proc. Natl. Acad. Sci. USA* **78**, 1001–1003 (1981).
11. L.-J. Wu, G. Randers-Pehrson, A. Xu, C. A. Waldren, C. R. Geard, Z. Yu and T. K. Hei, Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. *Proc. Natl. Acad. Sci. USA* **96**, 4959–4964 (1999).
12. R. G. Stevens, J. E. Morris and L. E. Anderson, Commentary: Hemochromatosis heterozygotes may constitute a radiation-sensitive subpopulation. *Radiat. Res.* **153**, 844–847 (2000).
13. H. Zhou, G. Randers-Pehrson, C. A. Waldren, D. Vannais, E. J. Hall and T. K. Hei, Induction of a bystander mutagenic effect of alpha particles in mammalian cells. *Proc. Natl. Acad. Sci. USA* **97**, 2099–2104 (2000).
14. G. Iliakis, G. E. Pantelias, R. Okayasu and W. F. Blakely, Induction by  $\text{H}_2\text{O}_2$  of DNA interphase chromosome damage in plateau-phase Chinese hamster ovary cells. *Radiat. Res.* **131**, 192–203 (1992).
15. L. L. de Zwart, J. H. N. Meerman, J. N. M. Commandeur and N. P. E. Vermeulen, Biomarkers of free radical damage: Applications in experimental animals and humans. *Free Radic. Biol. Med.* **26**, 202–226 (1999).

#### DISCUSSION

Antone L. Brooks

Washington State University at Tri-Cities, Kennewick, Washington

“Evaluation of Disease Risk” was a logical follow-up to the final paper in the session on “Uncertainties in Exposure and in Response”, where Dr. Harvey Mohrenweiser discussed the use of biomarkers of dose and reviewed markers of exposure. In our session, the theme was expanded to include markers of sensitivity and disease. The papers presented illustrated the relative role of genetic background in radiation sensitivity and the impact of genetic sensitivity on risk from radiation-induced disease.

Dr. Ken Kraemer of the National Institute of Health presented “Predisposition, Susceptibility and Radiation-induced Cancer”, which reviewed the role of sensitivity in radiation-induced disease. This talk illustrated a very good example of a genetic condition that alters sensitivity and response to an environmental insult. Dr. Kraemer discussed the role that ultraviolet (UV) radiation plays in the induction of skin cancers (basal cell carcinoma, squamous cell carcinoma, and melanoma) in individuals with the rare genetic disease xeroderma pigmentosum (XP). Those with this disease have more than a 1000-fold increase in risk for the development of these cancers. For XP, the link between UV-radiation exposure and the development of cancer is well established. However, unlike the other skin cancers, the induction of melanoma is not limited to areas where there is sunlight and UV-radiation exposure. This observation suggests additional mechanisms for the induction of melanoma. Dr. Kraemer also described the biochemical basis for this genetic deficiency and demonstrated that DNA repair deficiencies play a central role in development of the disease. Finally, he discussed the fact that those with this disease are not sensitive to exposure to ionizing radiation. This observation suggests very different pathways for cancer induced by ionizing radiation and UV radiation and supports the concept that the types of damage induced by ionizing radiation and the repair of that damage are unique. His talk demonstrated how an understanding of the mechanisms underlying disease can be used in establishing the impact of genetic background on risk in individuals with the disease. Such understanding makes it possible to predict sensitivity to environmental agents that have similar modes of action and illustrates that sensitivity to one agent does not necessarily confer sensitivity to exposure to other agents.

Dr. K. Sankaranarayanan from Leiden University presented the second talk, “Cancer Predisposition, Radiosensitivity and the Risk of Radiation-Induced Cancers: Biological Aspects and Computational Modeling”, in which he presented models that have been developed to define the impact

of genetic disease on cancer risk. These models made it possible to vary both the frequency of the genes in the population and the sensitivity of the carriers that are either homozygous or heterozygous for the genes associated with the disease. The models can predict the impact of genetic background on risk from low levels of radiation exposure. They also compared populations that were very homogeneous with respect to both cancer predisposition and radiosensitivity to populations that were more heterogeneous with respect to these traits. The models suggested that the more heterogeneous population would have a higher relative risk than the homogeneous population. The dose dependence was predicted to be nonlinear with a decrease in relative risks at higher doses. Dr. Sankaranarayanan did an excellent job of showing the magnitude of the impact of genetic disease, i.e. reduced DNA repair, on cancer risk. He demonstrated that the presence of a sensitive subpopulation would have very little impact on total cancer risk for genetic diseases that have low gene frequency, less than 10%. He also demonstrated that the relative risk for a total population would be affected only when there were very marked increases in cancer susceptibility and sensitivity (>1000-fold) in the individuals that had the sensitive phenotype. The models all assumed complete penetrance of the genes. Any decrease in penetrance, which is present for most genes studied, would decrease the risk. These results support the concept that increases in cancer risk related to genetic background will be detectable epidemiologically only when both the mutant alleles are common and the strength of the predisposition and radiosensitivity differentials are very dramatic. To date, we do not have examples of genetic diseases that have both a high frequency and high sensitivity. This presentation represented a very careful and complete review of genetic markers of both sensitivity and disease.

The final presentation in this session was "Hemochromatosis May Increase the Sensitivity to Radiation-Induced Carcinogenesis" by Dr. Richard G. Stevens of the University of Connecticut Health Center. The genes that predispose individuals to hemochromatosis have been identified, located on a specific chromosome, and sequenced. The frequency of the gene in carriers has been shown to be about 10%. Iron was postulated to act as a "food" for cancer cells or to produce free radicals that increase oxidative stress. The major emphasis of the presentation was on the role that iron plays in free radical production. Dr. Stevens reviewed a number of different epidemiological studies that suggest that carriers of this gene and persons who are homozygous for the hemochromatosis gene have an increased risk for cancer, especially in the liver. He also demonstrated that individuals who are homozygous for the gene have iron overload in many tissues and that this overload can be reduced with treatment. When the overload is decreased, the risk for cancer was also decreased. This suggests that the presence of the iron in the diet and in the organs plays a direct role in the development of cancer. However, the link between radiation sensitivity and hemochromatosis is presently limited to *in vitro* studies. Dr. Stevens reviewed studies currently under way at Pacific Northwest National Laboratory in which the level of iron in the diet, the genetic makeup of the mice, and the exposure to radiation are being combined to determine if identification of this gene could represent a marker of sensitivity. If these studies demonstrate an increased sensitivity to radiation, this gene could meet the criteria set out by Dr. Sankaranarayanan for major impact on risk. Hemochromatosis genes occur at high frequency and may have an increased sensitivity to radiation. If a high sensitivity is confirmed for hemochromatosis, this disease could have a major impact on cancer frequency.

In summary, the presentations in this session demonstrated how biological markers of radiation exposure and dose, markers of sensitivity, and markers of disease can be used to support and supplement current epidemiological approaches. As these cellular and molecular studies are continued, and as the techniques improve and become faster and more economical, biomarkers may play an important role in risk assessment. In the future, it may become possible to include genetic predisposition as a marker of radiation risk in the same way that age at exposure, sex, race, dose and economic status are currently used. Such molecular epidemiological approaches may become useful tools for understanding the mechanisms involved in the development of disease, and provide important information

on the magnitude of the health risk after exposure to low levels of man-made occupational and environmental radiation.

#### IV. TEMPORAL EFFECTS IN RADIATION EPIDEMIOLOGY—1

Chair: Bobby Scott

*Lovelace Respiratory Research Institute, Albuquerque, New Mexico*

#### Age-Time Distribution of Cancer Risks to be Expected from Acute or Chronic Exposures to General Mutagens

D. A. Pierce

*Radiation Effects Research Foundation, Hiroshima, Japan*

(with M. Vaeth, University of Aarhus)

Our aim is to indicate some mathematical considerations that may provide guidance in the descriptive analysis of excess cancer due to either acute or prolonged exposures to carcinogens. It is particularly challenging to carry out and interpret such analyses for prolonged exposures, and even for acute exposures it is difficult to distinguish between effects of age at exposure, time since exposure, and attained age. Carcinogenesis is quite complicated, and we prefer not to think of our formulation as a mathematical model for this. There are, however, some plausible aspects that allow interesting mathematical development that bear on the anticipated general character of age-time patterns of effects due to a general mutagen. This is based simply on the notion that a cancer is due to accumulation of mutations in a stem cell, and that a general mutagen might increase all their rates. The most notable aspect of the results is provision of possible insights into age-time patterns of risk rather independently of parameter values in the mathematical formulation.

Suppose, certainly as an idealization, that background cancer arises substantially as follows. There is a class of relevant mutations that can occur in a stem cell, each of which occurs at a rate that is independent of age but—importantly—may depend arbitrarily on which mutations have already occurred in the cell. A cell becomes malignant when some  $k$  of these mutations have occurred. For the moment, we equate the existence of a malignant cell with a cancer. Such a process is both biologically more specific and mathematically more general than the Armitage-Doll multistage model, but it remains true that background cancer rates would, for suitably small mutation rates, increase approximately as age to the  $k - 1$  power.

Now suppose that when a cell is exposed to a general mutagen at age-specific rate  $d(a)$ , the rates of all the mutations are increased by a factor  $[a + \beta d(a)]$ . This departs markedly from the usual considerations of the effects of a specific carcinogen in the Armitage-Doll multistage model, where a tenet has been that the carcinogen can affect only one or two of the stage transitions, in terms of the order of their occurrence.

It can be shown that under the above assumptions the result of exposure is to change the age scale for a cell from  $a$  to  $[a + \beta D(a)]$ , where  $D(a)$  is the cumulative dose by age  $a$ . It then follows directly that under this formulation the relative risk of an exposed to unexposed collection of cells is given by

$$RR(a) = \left[ 1 + \beta \frac{D(a)}{a} \right]^{k-1} [1 + \beta d(a)]. \quad (1)$$

The first term is the ratio of  $[a + \beta D(a)]^{k-1}$  to  $a^{k-1}$ , and the second term is a differential element resulting from the change of age scale. One could in this entire development consider  $d(a)$  as any given nonlinear function of dose rate, with  $D(a)$  the integral of this function.

Others have suggested the change of age scale argument independently of the specific considerations here. Note that, provided background cancer increases as a power of age, for whatever reason, Eq. (1) follows from the change of age scale assumption without regard to the development here.

However, it is significant that in our formulation the final term in Eq. (1) corresponds to the effect of the exposure causing the final one of the required mutations. Moreover, the terms in the expansion of the initial term in Eq. (1) correspond to the exposure causing 0, 1, 2, . . . mutations in the same cell. Although for  $RR(a)$  to involve a high-degree polynomial in dose may seem implausible, it should be observed that the function  $(1 + x)^p$  is very nearly linear in  $x$  even for  $p = 5$ , say, over a range where the function value is no greater than about 3 or 4—a fairly large relative risk in radiation studies. Moreover, because of this, the age patterns of risk given by Eq. (1) are insensitive to variations in the value of  $k$ , provided that corresponding adjustments are made to  $\beta$ .

When allowing for the time between the presence of a malignant cell and a cancer, both rates forming the ratio  $RR(a)$  are lagged and smoothed by a random latent period. The smoothing is fairly important, particularly in regard to the final factor of Eq. (1) when the exposure period is short. Applying this smoothing to  $RR(a)$  rather than the rates is more transparent and usually results in negligible error. The chance that a malignant cell may not develop into a cancer is important, affecting equally the two cancers involved but canceling in the relative risk.

The formula of Eq. (1) corresponds more closely than perhaps it should, given the idealization involved, to age–time patterns of risk due to both ionizing radiation and cigarette smoking. The ideas leading to it were first developed for the atomic bomb survivors (1). In this case the exposure was acute and follow-up began several years after exposure, leading to the simplification that during follow-up  $RR(a) = [1 + \beta D/a]^{k-1} = 1 + (k-1)\beta D/a + \dots$ , where  $D$  is simply the dose. For solid cancers, the  $RR$  is small enough for the indicated linear approximation in  $D$  to be totally adequate. If the decline in  $RR$  normally attributed to age at exposure is attributed to attained age  $a$ , then this is fundamentally the age–time pattern seen for most solid cancers in the atomic bomb survivor data. The suggestions that age at risk—as opposed to age at exposure or time since exposure—may be the primary age–time scale and that there is a simple reason why the  $RR$  may decrease with age indicate how such mathematics may be useful for descriptive analyses and interpretation of data. For cancers with strong hormonal influences, such as breast and thyroid, there are, not surprisingly, departures from predictions based on age alone.

For miners exposed to radon, matters are substantially more interesting, since as noted the descriptive analyses and their interpretation are quite challenging. From the joint analysis of a large number of cohorts, with far more information than is usually available, it has been possible to describe the age–time patterns of risk in some detail (2). These descriptions involve varying weights for dose according to time since exposure, a general decrease with attained age, and a possible effect of dose rate or duration of exposure. The characteristic age–time pattern for  $RR(a)$  resulting from such descriptions is a rather sharp increase beginning about 5 years after the start of exposure, continuing until about 5 years after exposure ends, then a decrease which is correspondingly rapid for 10–15 years and subsequently becomes more modest. Although the descriptive models are rather complex, the formula of Eq. (1) predicts essentially the same age–time patterns for various exposure scenarios and up to moderate doses. The risks for miners at high doses are large enough for Eq. (1) to predict modest upward curvature in dose, but as with the atomic bomb survivors, even linear models overpredict the risk at highest doses. Equation (1) may, to some extent, clarify the issue of the so-called inverse dose-rate effect, mainly through the impact of the final factor in that equation during a lengthy exposure period. More generally, since the observed  $RR$  varies markedly with age in relation to the exposure period, as described above, issues such as a dose-rate effect, and indeed the shape of the dose response, become quite complicated.

Although there is probably less reason than for ionizing radiation to think of cigarette smoking products as general mutagens, Eq. (1) also predicts the age–time patterns of lung cancer risk associated with cessation of smoking remarkably well. In both this setting and that for the underground miners, the final factor in Eq. (1) plays a major role through contributing substantially during the exposure period but vanishing after termination of exposure.

Important questions pertain to the value of such mathematics, and modeling of mechanisms more generally, in substantive analyses and interpretation of data. Most would agree that some hybrid approach of em-

pirical description and idealized deductive reasoning is the best aim. Almost never would one want to use something as simple as Eq. (1) for descriptive purposes. But there is need for guidance in descriptive analyses, which are not trivial even for acute exposures and become truly daunting for prolonged exposures. Seldom is there the opportunity for the heroic efforts employed during the past 15 years on joint analyses of the data for miners exposed to radon. Use of ideas indicated here could substantially affect analyses in more typical prolonged-exposure studies. However, the most important issues often involve interpretation of descriptions, where one is inevitably faced with the need to sort out plausible causality in the presence of too many highly correlated age–time–dose covariables. Considerations here have affected our attitude toward age-at-exposure effects for the A-bomb survivor data, and they could bring into question the current emphasis on time-since-exposure effects in interpreting the data for the miners.

#### References

1. D. A. Pierce and M. L. Mendelsohn, A model for radiation-related cancer suggested by the atomic bomb survivor data. *Radiat. Res.* **152**, 642–654 (1999).
2. National Research Council, Committee on the Biological Effects of Ionizing Radiation, *Health Effects of Exposure to Radon (BEIR VI)*. National Academy Press, Washington, DC, 1999.

### Multistage Models and the A-Bomb Survivor Data: Implications for Carcinogenic Mechanisms?

Suresh H. Moolgavkar

*Fred Hutchinson Cancer Research Center, Seattle, Washington*

This presentation consists of two parts. The first discusses the concept of multistage carcinogenesis in mathematical terms. The observation that both mutation and cell proliferation kinetics play essential roles in carcinogenesis suggests the framework for a mathematical model for carcinogenesis that incorporates the essential biological features of the process. The first multistage models for carcinogenesis (1) were proposed to explain the epidemiological observation that the age-specific incidence curves of many common human carcinomas rise roughly with some power of age. These early models ignored cell proliferation kinetics. Later models, e.g. the two-stage clonal expansion model (2), explicitly introduced stochastic birth and death processes to describe the kinetics of intermediate cell populations. It can be shown rather generally that the hazard functions arising from multistage models can be written as follows:  $h(t) = \mu_n E[X_n(t)|M(t) = 0]$ , where  $t$  is age (time),  $E$  denotes the expectation,  $\mu_n$  is the rate of the last mutation on the pathway to cancer,  $X_n$  is a random variable representing the number of cells in the premalignant compartment, and  $M$  is a random variable representing the number of malignant cells. Thus  $h(t)$  is the product of the last mutation rate and a conditional expectation. A commonly used approximation assumes that  $h(t) \sim \mu_n E[X_n(t)]$ ; i.e., the conditional expectation is replaced by the unconditional expectation. Additionally, most applications of the Armitage-Doll multistage model make one more approximation: Only the first non-zero term in the Taylor series expansion of the unconditional expectation is retained. With these two approximations, the hazard function of the Armitage-Doll model becomes  $h(t) \sim ct^k$ , where  $c$  and  $k$  are constants. The use of approximate solutions (3) can yield misleading results.

Analyses of the A-bomb survivor data using a number of versions of multistage models, undertaken in collaboration with Heidenreich, Luebeck and Hazelton, were motivated by a recent paper by Pierce and Mendelsohn (3). Based on a statistical smoothing of the data, they concluded that excess cancer rate after radiation exposure depends only on age and not on age at exposure. They ask, “Can this observation help to discriminate between various models of radiation carcinogenesis? Are there general implications regarding mathematical models of carcinogenesis?” Us-

ing an approximate form of the multistage model of Armitage and Doll, they contend that the observations are consistent with a multistage process if and only if exposure to radiation causes the same proportionate increase in each of the transition rates. Previously, we (4) had analyzed the data using the two-stage clonal expansion model and concluded that the data are consistent with radiation affecting only the initiation step. We have now used a number of models: the Pierce-Mendelsohn model, the two-stage clonal expansion model, the exact Armitage-Doll model, the exact Nordling model, and the attained age model. These analyses indicated that, among females, all the models fit about equally well as judged by the likelihood. Among males, however, the exact versions of the Armitage-Doll and Nordling models and the two-stage clonal expansion model fit better than the Pierce-Mendelsohn model. Moreover, these models predict very different patterns of excess risk, indicating that smoothing of data may yield misleading results.

#### References

1. P. Armitage and R. Doll, The age distribution of cancer and a multistage theory of carcinogenesis. *Br. J. Cancer* **8**, 1–12 (1954).
2. H. Moolgavkar and A. G. Knudson, Jr., Mutation and cancer: A model for human carcinogenesis. *J. Natl. Cancer Inst.* **66**, 1037–1051 (1981).
3. D. A. Pierce and M. L. Mendelsohn, A model for radiation-related cancer suggested by atomic bomb survivor data. *Radiat. Res.* **152**, 642–654 (1999).
4. M. Kai, E. G. Luebeck and S. H. Moolgavkar, Analysis of the incidence of solid cancer among atomic bomb survivors using a two-stage model of carcinogenesis. *Radiat. Res.* **148**, 348–358 (1997).

### Using a Biologically Motivated Cancer Model to Understand Dose and Temporal Radiation Effects

H. P. Leenhouts

National Institute of Public Health and the Environment (RIVM),  
Bilthoven, The Netherlands

(with M. J. P. Brugmans, RIVM)

In the last few years, we have developed a two-mutation clonal expansion model for radiation-induced carcinogenesis to function as a link between radiation effects at the cellular level and excess cancer incidence (1). Essential for the model is the assumption that two mutations are necessary for making a normal cell malignant and leading to cancer. The number of cells in the intermediate stage, with only one mutation, may increase in time at an expansion rate of  $\epsilon$ . Radiation is assumed to induce cancer by increasing the mutation rates,  $\mu_1$  and  $\mu_2$ , depending on the type of radiation and of cancer, using the dose relationship below derived from cellular radiation biology:  $\mu_i = (\mu_{bg,i} + a_{1,i} D + a_{2,i} D^2) \exp(-b_{1,i} D - b_{2,i} D^2)$ , where  $i$  represents the mutation rate number,  $\mu_{bg,i}$  the background mutation rate without exposure,  $D$  the dose in the considered model period, and  $a_{i,j}$  and  $b_{i,j}$  parameters to be fitted by the model.

This model simultaneously describes the dose and age dependence of radiation-induced cancer and has been used successfully for a number of cancers in animals and humans (1–4). Here we present some dose and time dependences that are relevant to the extrapolation of radiation effects found at high doses to the risks at low doses.

#### Effect of Dose Rate in Low-LET Radiation

For low-dose-rate exposure to low-LET radiation, a smaller radiation effect is observed for such effects as cell killing and mutation induction than for acute irradiation. This effect is caused by a smaller contribution of the quadratic component of the linear-quadratic dose–response relationship, which can be ascribed to repair of sublethal damage (5). The reduction of the radiation effect occurs for dose rates between about 1 Gy min<sup>-1</sup> and 0.1 Gy h<sup>-1</sup> (exposure periods of 1 min and a few hours).

For still lower dose rates, no further reduction is possible, resulting in a linear dose–response relationship.

Although it is assumed in the model that in principle both mutations may be dependent on radiation, for short exposures (say within a day) only the radiation effect of one mutation rate is important in the cancer incidence. In such a case the cellular dose–response relationship is reflected as for radiation-induced cancer incidence. This conclusion is illustrated by the experimental data of Coggle as described in ref. (1): The induction of lung tumors at age 15 months in mice exposed at a high dose rate at age 3 months resembles the (cellular) dose–effect relationship of the first mutation rate. The reduction in effect by lowering the dose rate can be observed in the dose–effect relationship for tumor induction.

These results show the dose-rate effect for low-LET radiation to have an important cellular component. This lesser effect at low dose rates, for example, is pertinent for evaluating cancer in the Japanese atomic bomb survivors, where the exposure occurred in a split second.

#### The “Reverse Dose-Rate” Effect for High-LET Radiation

The dose-rate effect as described above does not occur for high-LET radiation ( $\alpha$  particles and neutrons), because the quadratic component of the cellular dose–effect relationship is not significant for high-LET radiation. However, two other important aspects of the carcinogenesis model define the dose and time dependence for high-LET radiations:

1. The effect of the age at exposure. Depending on the parameters of the model, the radiation effect is dependent on the age at exposure. For example, for lung cancer, exposure of individuals around the age of 10 results in the highest cumulative incidence per unit dose at the age of 75. Exposure of older individuals is less effective since the time for development of the cancer is shorter. It is obvious that on the basis of this effect, two different exposures may show a reverse dose-rate effect; e.g., a dose received between 20 and 50 years of age has an effect about three times as large as the same dose received (with a higher dose rate) between 40 and 50 years of age.
2. The influence of cell killing. For high doses, the exponential part of Eq. (1) becomes significant. This results in a downward bending of the dose–effect relationship, indicating a greater effect per unit dose at lower doses than at high doses. Such a dose–response relationship has been observed for lung cancer in uranium miners and bone cancer in radium dial painters (3).

These aspects play a role in low-LET radiation as well, but the importance is overshadowed by the regular dose-rate effect. It should also be kept in mind that the incidence of radiation-induced cancer is dependent on both the age at exposure and follow-up time. This is especially important when comparing results from different epidemiological studies.

The model results show dose–response relationships for lung cancer after exposure to radon and bone cancer after radium intake, both of which may lead to a reverse dose-rate effect. The effect for high-LET radiation, which is completely different from the regular dose-rate effect for low-LET radiation, is expressed for much longer exposure times. It can be explained by a different effect for the age at exposure and/or radiation-induced cell killing.

#### Absolute Compared to Relative Radiation Risks

The two-mutation clonal expansion model assumes that radiation induces cancers in excess of the background or baseline cancer incidence. This is a direct consequence of Eq. (1). The effect of a short exposure to radiation is an increase in cumulative cancer incidence rather shortly after the exposure; this increase remains, in general, in excess of the background cancer incidence at older ages. However, relative to the background cancer incidence, the radiation-induced cancer incidence increases, reaches a maximum, and then decreases, mainly because the background incidence increases exponentially with age. Although the coefficients used in the two-mutation clonal expansion model are assumed to

be independent of age, for exposed children, the maximum (plateau) of the observed excess relative effect occurs sooner after exposure and is higher for persons exposed at older ages. This phenomenon is important for epidemiological investigations, where, in general, relative effects are observed. For example, a high relative effect for exposure at young age was observed for thyroid cancer in children rather soon after exposure to  $^{131}\text{I}$  from the reactor involved in the Chernobyl accident (3). For adults the maximum is lower for the same dose and will occur later.

### Conclusions

The two-mutation clonal expansion model, which can be considered to function as a bridge between cellular effects and cancer, has been successfully fitted to radiation-induced cancer for different organs and radiation types. It is important that the cellular dose–time–effect relationships are carefully implemented in the model. Experimental and epidemiological data support the implications. The model can be considered useful for the extrapolation of epidemiological radiation effects to radiation risks at low doses. Although the model predicts, in general, a linear dose–effect relationship at low doses, the additional dose and time dependences described by the model form an important improvement for the estimation of radiation risks from epidemiological data. The model also explicitly describes the radiation effect projected over the entire lifetime and, as such, provides a means to extrapolate to the entire expected radiation effect.

### References

1. H. P. Leenhouts and K. H. Chadwick, A two-mutation model of radiation carcinogenesis: application to lung tumours in rodents and implications for risk evaluation. *J. Radiol. Prot.* **14**, 115–130 (1994).
2. H. P. Leenhouts, Radiation-induced lung cancer in smokers and non-smokers: risk implications using a two-mutation carcinogenesis model. *Radiat. Environ. Biophys.* **38**, 57–71 (1999).
3. H. P. Leenhouts, M. J. P. Brugmans and K. H. Chadwick, Analysis of thyroid cancer data from the Ukraine after “Chernobyl” using a two-mutation carcinogenesis model. *Radiat. Environ. Biophys.* **39**, 89–98 (2000).
4. H. P. Leenhouts and M. J. P. Brugmans, An analysis of bone and head sinus cancers in radium dial painters using a two-mutation carcinogenesis model. *J. Radiol. Prot.* **20**, 169–188 (2000).
5. H. P. Leenhouts and K. H. Chadwick, The influence of dose rate on the dose–effect relationship. *J. Radiol. Prot.* **10**, 96–102 (1990).

## The Importance of Promotion in Lung Carcinogenesis for Protracted Exposures to Radon and Radon Progeny

E. G. Luebeck

*Fred Hutchinson Cancer Research Center, Seattle, Washington*

(with S. B. Curtis, W. D. Hazelton and S. H. Moolgavkar, Fred Hutchinson Cancer Research Center)

Recent analyses of lung cancer mortality in the Colorado uranium miners cohort (1) and the Chinese tin miners cohort (Hazelton *et al.*, manuscript submitted) using the two-stage clonal expansion model led to the startling conclusion that promotion of intermediate cells (cells that have suffered the first rate-limiting step toward malignancy) in response to exposures to radon and radon progeny totally dominates radon-induced initiation of these cells. Because promotion is critically dependent on duration of exposure, the derived radiation risks are subject to “protraction effects” which are distinct from the traditional “dose-rate” effects. Such protraction effects provide an alternative to the “sensitive window in the cell cycle” hypothesis for the inverse dose-rate effect seen in epidemiological studies involving exposures to high-LET radiation (2).

Based on the parameter estimates obtained from the analysis of the Colorado uranium miners cohort, we have investigated the effects of age,

age at start of exposure, and duration (dose protraction) on lung cancer risks. The two-stage clonal expansion model provides a plausible explanation for the observed inverse dose-rate effect, defined here as a relative increase in the lifetime risk of dying from lung cancer when a given total dose is protracted over time. This effect is commonly observed in occupational studies involving miners who were exposed to relatively high doses of  $\alpha$ -particle radiation and in the context of the two-stage clonal expansion model can be attributed to the predominance of the promotion process over the initiation process.

However, the model can also be used to study protraction effects at very low exposures rates, much lower than the levels typically encountered in mines. When extrapolating radon exposures and exposure rates to levels relevant to the indoor radon problem, the model predicts that the inverse dose-rate effect is attenuated. Yet, compared with acute exposures (<1 h), highly protracted exposures (>1 month) may increase the lifetime excess absolute risk more than 10-fold. In contrast, in the absence of promotion, as may be the case with low-LET radiation (3), the model also predicts a direct dose-rate effect in the case when the exposure is protracted over time.

Whether or not the predicted promotional response is due to ambient co-carcinogens (uranium ore dust, fossil fuel exhausts) in the mines or to a direct promotional effect in premalignant lesions exposed to  $\alpha$  particles cannot be decided on the basis of the epidemiological data analyzed here. However, a reanalysis of the Pacific Northwest National Laboratory rat study (4) suggests that uranium ore dust may play a promotional role in tumorigenesis of the rat lung.

Other explanations for the promotional effect of radon may be given. For instance, it is possible that  $\alpha$  particles may trigger so-called bystander effects that may cause cell proliferative responses through changes in gene expression and/or disruption in cell-to-cell signaling. However, these phenomena are usually seen in *in vitro* studies involving acute irradiations. Alternatively, we may think of initiated cells as stem cells that have lost the ability to maintain proper homeostasis. In the presence of radiation-induced cell killing, these cells overcompensate for cell loss among their differentiated descendants through an occasional switch from asymmetric cell division to symmetric cell division, increasing net cell proliferation. This mechanism may not require direct exposure of initiated cells to  $\alpha$  particles but could be controlled by some “action at a distance”, that is, may be mediated by cellular signals that are inappropriately interpreted by the initiated stem cell.

We hope that these questions can be answered by new studies and experiments designed to explicitly test these hypotheses.

### References

1. E. G. Luebeck, W. F. Heidenreich, W. D. Hazelton, H. G. Paretzke and S. H. Moolgavkar, Biologically based analysis of the Colorado uranium miners cohort data: Age, dose and dose-rate effects. *Radiat. Res.* **152**, 339–351 (1999).
2. D. J. Brenner, The significance of dose rate in assessing radon exposure. *Health Phys.* **79**, 76–79 (1994).
3. S. H. Moolgavkar, Stochastic cancer models: Application to analyses of solid cancer incidence in the cohort of A-bomb survivors. *Nucl. Energy* **36**, 447–451 (1997).
4. E. G. Luebeck, S. B. Curtis, F. T. Cross and S. H. Moolgavkar, Two-stage model of radon-induced malignant lung tumors in rats: Effects of cell killing. *Radiat. Res.* **145**, 163–173 (1996).

## DISCUSSION: Temporal Effects in Radiation Epidemiology

Daniel Krewski

*University of Ottawa, Ottawa, Ontario, Canada*

As with other carcinogenic agents, temporal patterns of exposure and risk are important in understanding radiation carcinogenesis. To characterize the effects of exposure at different ages, Murdoch and Krewski (1)

introduced the concept of “relative effectiveness” of exposure at different ages using both the Armitage-Doll multistage model and the Moolgavkar-Venzon-Knudson two-stage clonal expansion model of carcinogenesis. Analytical results for these two models showed that the relative effectiveness of exposure at different ages depends on which stage of the model is affected: In general, exposures at younger ages are more effective than later exposures when an early stage in the process of malignant cellular transformation is affected. Agents that increase the rate of proliferation of intermediate cells also have an important impact on temporal patterns of risk. This analysis demonstrated that the use of a lifetime average daily dose to predict lifetime risk could lead to both overestimation and underestimation of risk, depending on which stages of the model are affected by exposure. However, using the relative effectiveness function, it is possible to define a weighted average lifetime equivalent constant dose that leads to the correct lifetime risk. Goddard *et al.* (2) subsequently demonstrated how the relative effectiveness function can be used to describe temporal patterns of exposure and risk using a series of examples involving chemical carcinogenesis.

Building on initial work by Pierce and Mendelsohn (3), Pierce and Vaeth (4) proposed a general mutagen model to describe the temporal characteristics of radiation cancer risks. This model is based on the assumptions that cancer is caused by mutations accumulating in stem cells throughout the course of a lifetime, and that radiation is a general mutagen that can cause virtually any of these mutations. Radiation is assumed to have the same multiplicative effect on all transition rates. Cell proliferation is indirectly incorporated as a consequence of cumulative mutations.

The general mutagen model implies that background cancer incidence rates increase as a power of age (specifically,  $\text{age}^{k-1}$ , where  $k$  is the number of mutations), as is the case in the Armitage-Doll model. The excess absolute risk does not depend on age at exposure, but increases as  $\text{age}^{k-2}$ . For acute exposures, the excess relative risk, ERR, decreases in proportion to the reciprocal of age. As with other biologically motivated models, age at exposure, time since exposure, and attained age can be incorporated into the model in a natural temporal manner. In comparison with the empirical model preferred by the BEIR VI committee for radon and lung cancer (5), the general mutagen model predicted somewhat lower ERRs at younger ages and notably higher ERRs at older ages.

Moolgavkar (6) used the two-stage clonal expansion model to describe temporal patterns of cancer risk among atomic bomb survivors. In addition to two critical mutations, the role of both tissue growth and cell kinetics in malignant transformation is explicitly considered. The exact form of the model is preferred to the approximate form since the hazard functions differ qualitatively: Specifically, the hazard for the exact form of the model is bounded and returns to baseline levels when exposure ends. Duration of exposure is more important for promoters that increase cell proliferation rates than for initiators that increase the first stage mutation rate. Although the effect of increased cell proliferation is to increase the hazard function early on, it is possible that the hazard may be reduced relative to background at later ages.

The two-stage clonal expansion model predicts the inverse dose-rate effect due to promotion for radon-induced lung cancer (7) and the direct dose-rate effect seen in the A-bomb survivor data (8). Leenhouts and Brugmans (9) further noted that the two-stage clonal expansion model produces curvilinear or linear exposure-response relationships, respectively, with acute or chronic exposure to low-LET radiation. The model can be extended to more than two stages (10), with the highest likelihood for colorectal cancer achieved with a four-stage model. Although the second-stage mutation rate was not significantly affected by radiation in these analyses, an ongoing analysis of radon and lung cancer in Chinese tin miners suggests that the second-stage mutation rate is also important (11). Although the inverse dose-rate effect for radon predicted by the two-stage clonal expansion model in this latter analysis is attenuated at low doses, protracted exposures may notably increase the lifetime excess absolute risk. This analysis illustrates how the roles of initiation and promotion in protraction enhancement can be reasonably explained by the two-stage clonal expansion.

This work demonstrates that applications of biologically motivated models of radiation carcinogenesis such as the general mutation and two-

stage clonal expansion models are becoming more commonplace. These models provide important insights into temporal patterns of exposure and risk and related properties such as the inverse dose-rate effect. Although it is difficult to capture all of the complexities of radiation carcinogenesis in a formal biological model, such models stimulate thinking about the mechanisms by which ionizing radiation increases cancer risk, which can lead to testable hypotheses and further refinement of the models.

#### References

1. D. J. Murdoch and D. Krewski, Carcinogenic risk assessment with time-dependent exposure patterns. *Risk Anal.* **8**, 521–530 (1988).
2. M. J. Goddard, D. J. Murdoch and D. Krewski, Temporal aspects of risk characterization. *Inhal. Toxicol.* **7**, 1005–1008 (1995).
3. D. A. Pierce and M. L. Mendelsohn, A model for radiation-related cancer suggested by atomic bomb survivor data. *Radiat. Res.* **152**, 642–665 (1999).
4. D. A. Pierce, Age-time distributions of cancer risks to be expected from acute or chronic exposures to general mutagens. *Radiat. Res.* **154**, 727–728 (2000). [extended abstract]
5. National Research Council, Committee on the Biological Effects of Ionizing Radiation, *Health Effects of Exposure to Radon (BEIR VI)*. National Academy Press, Washington, DC, 1999.
6. S. H. Moolgavkar, Multistage models and the A-bomb survivor data: Implications for carcinogenic mechanisms. *Radiat. Res.* **154**, 728–729 (2000). [extended abstract]
7. E. G. Luebeck, W. F. Heidenreich, W. D. Hazelton, H. G. Paretzke and S. H. Moolgavkar, Biologically based analysis of the Colorado uranium miner cohort data: Age, dose and dose-rate effects. *Radiat. Res.* **152**, 339–351 (1999).
8. M. Kai, E. G. Luebeck and S. H. Moolgavkar, Analysis of the incidence of solid tumors among atomic bomb survivors using a two-stage model of carcinogenesis. *Radiat. Res.* **148**, 348–358 (1997).
9. H. P. Leenhouts, Using a biologically motivated cancer model to understand dose and temporal radiation effects. *Radiat. Res.* **154**, 729–730 (2000). [extended abstract]
10. S. H. Moolgavkar, M. Schwarz and D. Krewski, Mechanisms of carcinogenesis and biologically based models for the estimation and prediction of risk. In *Quantitative Estimation and Prediction of Human Cancer Risks* (S. H. Moolgavkar, D. Krewski, L. Zeise, E. Cardis and H. Moller, Eds.), pp. 179–237. Scientific Publications No. 131, IARC, Lyon, 1999.
11. E. G. Luebeck, The importance of promotion in lung carcinogenesis for protracted exposures to radon and radon progeny. *Radiat. Res.* **154**, 730–731 (2000). [extended abstract]

## V. TEMPORAL EFFECTS IN RADIATION EPIDEMIOLOGY—II

Chair: Heather Stockwell

*Department of Energy, Germantown, Maryland*

### Age and Time Patterns in Thyroid Cancer after the Chernobyl Accident in the Ukraine

W. F. Heidenreich

*GSF—National Research Center for Environment and Health, Institute for Radiation Protection, 85764 Neuherberg, Germany*

(with T. I. Bogdanova, Ukrainian Research Institute of Endocrinology and Metabolism; P. Jacob, GSF—National Research Center for Environment and Health; A. G. Biryukov and N. D. Tronko, Ukrainian Research Institute of Endocrinology and Metabolism)

After the Chernobyl accident, the number of cases of thyroid cancer among children and adolescents reported increased markedly in Belarus

and in the Ukraine. The time and age pattern of this increase in Belarus has been described in ref. (1). A corresponding analysis is presented here for the Ukraine, particularly the northern part, where the reactor is located. Children born in the years 1968 through 1997 were followed from 1986 to 1998. Risk coefficients for both countries were estimated in ref. (2).

Background rates in the Ukraine can be extracted from the early cases observed from 1986 through 1988, before substantial effects of the released radioactivity are expected, from the cases among children born after 1986, when most of the iodine-131 had already decayed, and from persons who lived in the southern, less contaminated areas of the Ukraine. For the comparison of thyroid cancer rates in northern and southern Ukraine, we used the same regions as in ref. (3). We estimated a (presumably too high) background risk function which depends on the product of a power of age and a linear function of the year of diagnosis of tumor. The latter term allows for screening effects and the possible increase in rates due to exposure to radiation. Poisson regression indicated an increase in the rate of thyroid cancer by a factor of 2 from 1986 to 1998. We call this risk function spo-south (where "spo" is spontaneous, i.e. background) as it is dominated by the observations from southern Ukraine. A second (presumably too low) estimate of background termed spo-Ukr uses the power of age from spo-south (to stabilize the function for the age range up to 30 years) times a number which is fitted to the early and late cases only.

While the observed cases for the Ukraine are a factor of 2.6 or less higher than the expected numbers, the excess in the north is much larger (factor of 4 for spo-south and factor of 7 for spo-Ukr). The excess number of cases (412 compared to 360) differs less. Therefore, we extract features of the excess absolute risk (EAR) function for northern Ukraine.

Most of the thyroid exposure occurred during the first month after the accident. Thus time since the accident is also time since exposure for our purposes. The observed cases suggest no effect for about 3 years and then a linear increase of EAR up to 1998. This dependence on time since exposure is in agreement with that found for Gomel (Belarus) up to 1995. The slope of the increase is changing with age at exposure; we estimated it for age-at-exposure intervals of 3 years with a minimum latent period of about 3 years. Compared to Gomel, the decrease in effect with age at exposure is less pronounced, and there is an increase in the oldest age-at-exposure group that is not found in Gomel.

The dependence of EAR on age at exposure is due in part to different doses received after the accident. Therefore, a more interesting quantity is EAR per dose. The dependence of dose on age at exposure and on sex has been extracted from about 60,000 direct measurements of thyroid activities that were performed in May and June 1986 (4). With the result from that work the age at exposure dependence of EAR per dose was calculated. It is roughly constant for age at exposure up to 15 years. We cannot confirm the opinion (5) that the thyroid is more sensitive to radiation dose for younger children (1–5 years of age) compared to older ones. For age at exposure 16 to 18 years, the EAR per dose is a factor of almost 2 larger than for the younger age-at-exposure groups. This is entirely due to the effect in females and is not found in Gomel.

The comparison of patterns of time since exposure and age at exposure in Belarus and Ukraine may help to locate problems in the data sets. Further work on the nature and size of thyroid cancers found is suggested in both countries.

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#### References

1. W. F. Heidenreich, J. Kenigsberg, P. Jacob, H. Buglova, G. Goulko, H. G. Paretzke, E. P. Demidchik and A. Golovneva, Time trends of childhood thyroid cancer in Belarus. *Radiat. Res.* **151**, 617–625 (1999).

2. P. Jacob, G. Goulko, W. Heidenreich, I. Likhtarev, I. Kairo, N. D. Tronko, T. I. Bogdanova, J. Kenigsberg, E. Buglova and I. Zvonova, Thyroid cancer risk to children calculated. *Nature* **392**, 31–32 (1998).
3. B. Sobolev, W. F. Heidenreich, I. Kairo, P. Jacob, G. Goulko and I. Likhtarev, Thyroid cancer incidence in the Ukraine after the Chernobyl accident: Comparison with spontaneous incidence. *Radiat. Environ. Biophys.* **36**, 195–199 (1997).
4. W. F. Heidenreich, I. Kayro, M. Chepurny, P. Jacob, V. Spak, G. M. Goulko and H. G. Paretzke, Age- and sex-specific relative thyroid exposure in Ukraine after the Chernobyl accident. *Health Phys.*, in press.
5. R. E. Shore and X. Xue, Comparative thyroid cancer risk of childhood and adult radiation exposure and estimation of lifetime risk. In *Radiation and Thyroid Cancer* (G. Thomas, A. Karaoglou and E. D. Williams, Eds.), pp. 491–498. World Scientific, Singapore, 1999.

### The Influence of Age at Exposure to Radiation on Cancer Risk in Humans

Steve Wing

*Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, North Carolina*

The J-shaped curve of human mortality suggests that frailty is generally highest at the extremes of the life span. Spontaneous abortion rates are highest in the first trimester of gestation (1), and death rates are highest in infancy and old age (2). The young and old experience heightened sensitivity to temperature extremes, infection, toxins and pharmacological agents.

Are the young and old also more susceptible to cancer caused by ionizing radiation? Epidemiological consideration of this question has been strongly influenced by studies of A-bomb survivors. The cancer experience of A-bomb survivors exposed *in utero* does not suggest especially high sensitivity of the fetus, and among adult survivors the relative risk per unit dose declines with age. In contrast, studies of childhood cancer in populations with low infant mortality show cancer effects of prenatal X rays at low doses, and several nuclear worker studies have shown greater risks at older than at younger ages of exposure. Stewart argues that the experience of A-bomb survivors gives a misleading impression about the late effects of radiation due to selection in favor of young adults with high levels of resistance in the aftermath of the nuclear blasts (3). According to Stewart, cancer risks from ionizing radiation are exceptionally high not only during organogenesis (due to rapid cell division) but also at older ages (due to lower immune system competence). According to other models of carcinogenesis, passage through earlier stages of mutagenesis and reduced effectiveness of cellular repair could also increase the susceptibility of older people to cancer caused by radiation (4, 5).

Empirical evaluation of the influence of age at exposure on radiation risk must take account of the time course of the disease process. For acute effects, age at exposure and age at risk are approximately coincident. In the case of chronic effects, ages at exposure and ages at risk may be separated by years or decades. Long intervals increase opportunities for selective survival and competing risks. Long intervals also require analytical differentiation of age at exposure from age at risk and consideration of ongoing age effects when exposures occur over a range of ages.

Evidence of increased radiation–cancer dose response for older-age radiation exposures has been described in cohort studies of nuclear workers at the Hanford, Oak Ridge and Fernald facilities and Santa Susana Field Laboratory and in a nested case–control study of multiple myeloma at four nuclear facilities (3, 6–9). Models that allow for differing dose–response relationships for younger and older ages at exposure fit better than models that do not. Although it can be assumed that changes in age-related sensitivity for individuals are gradual (individual risk from exposure changes as a smooth function of age), average population values

may be fitted comparably by smooth and step functions of age at exposure.

Use of step functions for age at exposure is analogous to exposure lagging. Doses within the critical age or time period are given a weight of one and other doses are given a weight of zero. This method permits models to be estimated with simultaneous consideration of exposures at different ages as covariates. However, because radiation exposures for most nuclear workers occur at low dose rates, compartmentalization of cumulative doses within ranges of age shifts the dose distribution within each age band to lower values. Dose-response estimates become more unstable as slopes are estimated over smaller ranges of dose.

Biases could influence the appearance of age effects. However, when dose response depends on age at exposure, additional time-related requirements are needed to support scenarios in which age effects could be artifacts of confounding or measurement error. For example, if exposure to another carcinogen were to account for age-at-exposure effects observed among nuclear workers, the exposure would have to be associated with dose at older ages but not at younger ages. Similarly, an age-related pattern of exposure misclassification would be required to explain age-at-exposure relationships. Because cancers caused by radiation exposure at older ages must occur, on average, at older ages than those caused by exposures at younger ages, the declining sensitivity and specificity of death certificate diagnoses at old ages could obscure increases in the radiation-cancer dose response with age. The fact that colinearity of multiple time-related factors in cohort studies usually results in the ability to produce similar levels of overall fit of the model with different combinations of time-related variables means that biological considerations must be used to choose the most plausible models.

Evidence from a number of nuclear worker studies suggests that older adults are more sensitive than younger adults to cancer caused by radiation. Studies of nuclear workers who have been monitored over long periods with personal dosimeters will increase in scientific value over the next decades. Longer follow-up, greater accumulation of deaths, and increased attention to problems of exposure measurement will increase opportunities for analyzing specific cancers, longer latencies, influences of age at exposure, and the roles of other exposures and aspects of susceptibility. Along with evidence of an effect of *in utero* exposure to diagnostic X rays on childhood cancer, these worker studies are consistent with the idea that sensitivity to cancer risk from radiation is greatest at the same periods of life in which sensitivity to other agents is greatest.

#### References

1. M. Goldhaber and B. Fireman, The fetal life table revisited: Spontaneous abortion rates in three Kaiser Permanente cohorts. *Epidemiology* **2**, 33–39 (1991).
2. D. Bogue, Demographic aspects of mortality. In *Principles of Demography*, pp. 548–559. Wiley, New York, 1969.
3. A. Stewart, The role of epidemiology in the detection of harmful effects of radiation. *Environ. Health Perspect.* **108**, 93–96 (2000).
4. D. Goukassian, G. Faten, M. Yaar, M. Eller, U. Nehal and B. Gilchrest, Mechanisms and implications of the age-associated decrease in DNA repair capacity. *FASEB J.* **14**, 1325–1334 (2000).
5. S-I. Moriwakim, S. Ray, R. Tarone, K. Kraemer and L. Grossman, The effect of donor age on the processing of UV-damaged DNA by cultured human cells: Reduced DNA repair capacity and increased DNA mutability. *Mutat. Res.* **364**, 117–123 (1996).
6. D. B. Richardson and S. Wing, Greater sensitivity to radiation exposures at older ages among workers at Oak Ridge National Laboratory: Follow-up through 1990. *Int. J. Epidemiol.* **28**, 428–436 (1999).
7. B. Ritz, Radiation exposure and cancer mortality in uranium processing workers. *Epidemiology* **10**, 531–538 (1999).
8. B. Ritz, H. Morganstern and J. Moncau, Age at exposure modifies the effects of low-level ionizing radiation on cancer mortality in an occupational cohort. *Epidemiology* **10**, 135–140 (1999).
9. S. Wing, D. Richardson, S. Wolf, G. Mihlan, D. Crawford-Brown

and J. Wood, A case control study of multiple myeloma at four nuclear facilities. *Ann. Epidemiol.* **10**, 144–153 (2000).

### Age-Time Patterns for Cancer and Noncancer Excess Risks in the Atomic Bomb Survivors

D. L. Preston

*Department of Statistics, Radiation Effects Research Foundation, Hiroshima, Japan*

(with D. A. Pierce and Y. Shimizu, Radiation Effects Research Foundation)

Among the most important findings to emerge from recent analyses of the Life Span Study (LSS) of people exposed to radiation from the atomic bombs in Hiroshima and Nagasaki are the indications that excess solid cancer rates appear to increase throughout life after exposure at any age (1) and the increasingly compelling evidence for radiation-associated long-term increases in noncancer disease mortality (2) and morbidity (3). Because of the size of the cohort (more than 86,000 people exposed to the bombs for whom dose estimates are available) and the length and completeness of follow-up, it is now possible to move from the relatively straightforward task of determining whether or not the data provide evidence of a dose response to the characterization of how these risks might depend on factors such as sex, age at exposure, age, and time since exposure.

In this presentation, we discuss the nature of the age and time patterns of the radiation-associated excess solid cancer and noncancer risks that are seen in the LSS cohort for the period from 1950 (1958 for solid cancer incidence) through the mid-1990s. We consider descriptions in terms of excess relative risks, excess rates, and estimates of the lifetime risks for radiation-associated death. The results make use of preliminary analyses of cancer and noncancer mortality data for the period from 1950 through 1995 and solid cancer incidence for the period from 1958 through 1994. More detailed descriptions and analyses of these data will be published elsewhere.

Most analyses and summaries of the LSS data on solid cancer focus on descriptions in terms of the excess relative risk (ERR). However, it is especially important when considering age-time patterns (and gender differences) to present descriptions in terms of both the ERR and the excess absolute rate (EAR) since, when variation with age and other factors are taken into account, these provide equally adequate descriptions of the data and complementary insights into the nature of excess cancer risks in exposed populations.

The most common solid cancer ERR model allows for variation with age at exposure and gender but considers the ERR to be constant with respect to age. Our most recent report on cancer mortality (1) indicates that the ERR for people exposed as children may be decreasing with time, while Kellener and Barclay (4) noted that a description in which the ERR varies with age at death (or cancer diagnosis) and little or no dependence on age at exposure is a useful alternative to the usual ERR model.

The limitations of simple ERR models for describing solid cancer risks are clearly indicated in the updated LSS data on solid cancer incidence, which extend the follow-up 7 years beyond the period considered by Thompson *et al.* (5). The new data set contains information on almost 11,700 first primary solid cancer cases with 2.3 million migration-adjusted person years over a 37-year follow-up period. We estimate that there have been about 760 radiation-associated cancer deaths over the study period. When all solid cancers are considered as a group, there is statistically significant variation in the ERR with age at exposure and attained age, and neither of these effects by itself can adequately describe the age-time variability in the ERR. In an ERR model including both age-at-exposure and attained-age effects, the attained-age specific ERRs decrease by about 24% per decade increase in age at exposure (95% CI 11%, 35%) while the ERR decreases in proportion to (attained age)<sup>1.4</sup>

(95% CI  $-2.1, -0.6$ ) after exposure at any age. This temporal pattern does not vary significantly by gender.

Care should be taken in interpreting descriptive associations such as those described above. Description of age–time patterns for all solid cancers is important, but different analyses may be required when attempting to distinguish between associations of risk with age–time variables and the biological effects of these variables. Not only may some types of cancer, e.g. breast and thyroid, have distinctive age–time patterns, but the distribution of solid cancer types differs greatly by sex, and sex-specific background rates have different age–time patterns. For example, Pierce and Mendelsohn (6) emphasized that, for a collection of the major non-sex-specific solid cancers, the ERR varies with age and not, after allowing for age, with age at exposure or time since exposure. Possible biological reasons for this were explored through mathematical modeling of mutations and cancer. The age–time patterns of interest in that paper, for the selected cancer types, are still seen with the extended follow-up data.

While the LSS solid cancer ERR may decrease with attained age, especially for those exposed as children, the EAR increases rapidly with age for all ages at exposure. The increase is roughly proportional to age-squared with age-specific rates tending to decrease with increasing age at exposure. There is a weak suggestion that excess rates increase more rapidly for men than for women. The excess rate estimates clearly suggest that, regardless of age at exposure, solid cancer rates are elevated throughout life after exposure and that the magnitude of the excess increases with age.

The age–time patterns in the excess risk for the latest LSS data on solid cancer mortality (with 8808 cancer deaths and almost 3 million person-years of follow-up) are similar to those seen in the incidence data. While the evidence for variation in the ERR with attained age after allowing for age-at-exposure effects is not as strong as in the incidence data, there is some evidence that the ERR for those exposed as children has decreased with attained age. Despite this decrease in the ERR after childhood exposure, excess solid cancer death rates are increasing similarly for all ages at exposure.

We recently published an extended report on radiation and noncancer mortality death rates in the LSS (2). The basic finding of that report is that there is a significant association between radiation dose and noncancer disease mortality rates in the LSS that cannot be explained on the basis of diagnostic misclassification, confounding or selection effects. Similar results are seen with the extended follow-up.

Under a linear dose–response model, risks are increased by about 10% after a dose of 1 Sv for all noncancer diseases as a group and for several major general categories of such diseases, including heart disease, stroke, respiratory diseases, and digestive diseases. Because the background rates are large and the relative increase is only modest, it is much more difficult to characterize the nature of the dose response or the temporal patterns of the excess noncancer disease risk. There is no indication of statistically significant variation in the noncancer disease ERR with sex, age at exposure, or time. However, because of the large background rate and relatively small excess risk, there is little power to detect what might be fairly large departures from the simple constant ERR model. In particular, point estimates of age-at-exposure (20% decrease per decade increase) and age effects (ERR increasing in proportion to age) are comparable in magnitude to those seen for the solid cancer ERR. The noncancer disease EAR exhibits a clear and highly significant increase with attained age. There is no evidence for statistically significant variation in the EAR with age at exposure, but the point estimate of the effect is not small (an increase of 25% per decade increase in age at exposure). After allowing for an age-at-exposure effect, the rate of increase in the EAR with age is proportional to age to the 7th power. There is no indication that the excess rates vary significantly by gender.

The most appropriate comparisons of excess noncancer and solid cancer risks in the LSS are made in terms of age-dependent EARs. These comparisons suggest that after a 1-Sv exposure prior to age 50 the excess noncancer and solid cancer rates are roughly comparable for attained ages of 60 to 80. The importance of this finding for radiation protection depends largely on the nature of the noncancer-disease dose response, which

as noted above remains uncertain. If the dose–response function were linear, then at this time in the LSS the number of radiation-associated solid cancer deaths is about half of that for solid cancer deaths; for a linear-quadratic noncancer dose response, this ratio decreases to about one-third. However, if we consider this comparison at a given low dose, e.g. 0.1 Sv, under a linear-quadratic model the estimated number of radiation-associated noncancer deaths is about 15% of that for solid cancer.

With the current follow-up data, it is interesting to compute estimates of the lifetime impact of the radiation exposure on the 50,000 LSS cohort members with dose estimates of 0.005 Sv (mean colon dose of 0.2 Sv) or more. Using current parameter estimates with allowance for attained-age and age-at-exposure effects on the solid cancer risks, we estimate that about 3% will die from radiation-associated solid cancers, 0.2% of radiation-associated leukemias, and 1.4% (1% under an LQ model) from radiation-associated noncancer disease causes. Uncertainties of the solid cancer and noncancer estimates are roughly 50%. The life expectancy for members of this group will be shortened by about 6 months.

#### References

1. D. A. Pierce, Y. Shimizu, D. L. Preston, M. Vaeth and K. Mabuchi, Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950–1990. *Radiat. Res.* **146**, 1–27 (1996).
2. Y. Shimizu, D. A. Pierce, D. L. Preston and K. Mabuchi, Studies of the mortality of atomic bomb survivors. Report 12, Part II. Noncancer: 1950–1990. *Radiat. Res.* **152**, 347–389 (1999).
3. K. Kodama, S. Fujiwara, M. Yamada, F. Kasagi, Y. Shimizu and I. Shigematsu, Profiles of non-cancer diseases in atomic bomb survivors. *World Health Stat. Q.* **49**, 7–16 (1996).
4. A. M. Kellerer and D. Barclay, Age dependences in the modelling of radiation carcinogenesis. *Radiat. Prot. Dosim.* **41**, 273–281 (1992).
5. D. E. Thompson, K. Mabuchi, E. Ron, M. Soda, M. Tokunaga, S. Ochiaikubo, S. Sugimoto, T. Ikeda, M. Terasaki and S. Izumi, Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. *Radiat. Res.* **137** (Suppl.), S17–S67 (1994).
6. D. A. Pierce and M. L. Mendelsohn, A model for radiation-related cancer suggested by atomic bomb survivor data. *Radiat. Res.* **152**, 642–654 (1999).

## DISCUSSION: Temporal Effects in Radiation Epidemiology—II

Ethel Gilbert

*National Cancer Institute, Bethesda, Maryland*

We have heard a group of interesting papers addressing age and time patterns in three very different settings. The A-bomb survivor study is one of the earliest and most influential studies. Subjects were exposed at all ages and have been followed for nearly 50 years, which makes the study a standout in its potential for addressing age and time effects. By contrast, exposure in the worker studies was primarily to adult males and was protracted over time, and doses that drive risk estimates were about an order of magnitude lower than doses that drive risk estimates from the A-bomb survivor studies. Studies of exposure from Chernobyl are still very young, with only about 12 years of follow-up, much shorter than either the A-bomb survivor or the worker studies. The study discussed here involved only subjects who were exposed under age 18.

In the paper on thyroid cancer in the Ukraine and Belarus, Dr. Heidenreich and coworkers have found evidence of an increase in the excess absolute risk (EAR) with time since exposure. Factors that might contribute to this increase are the increase in risk after a minimal latent period, the increase in baseline risk with attained age, and perhaps increased surveillance. With the current limited follow-up, it is difficult to say much more than this regarding time trends, or to predict future trends.

An objective in evaluating these data is to compare the time and age

patterns of thyroid cancer risks after exposure to  $^{131}\text{I}$  with those after external exposure. Although there was no evidence of an age-at-exposure effect in the Ukraine or Belarus data, it would be of interest to know if the data were compatible with an age effect of the magnitude found in external radiation studies.

Dr. Heidenreich has based his analyses on excess absolute risks because these are less dependent on the baseline risk. However, this may not be the ideal choice for comparing age and time trends with those identified in studies of external exposure and expressed in terms of excess relative risks (ERR). I have fitted an ERR model to the Belarus data presented in an earlier paper by Dr. Heidenreich and colleagues, and found that although the estimates of the overall relative risks were highly sensitive to the choice of baseline, the parameters describing the effects of age at exposure and time since exposure were much less sensitive to this choice.

Turning to Dr. Wing's paper, I will start by reviewing age-at-exposure effects in high-dose studies. It is fairly well established that at least in terms of the ERR for the first 20–30 years of follow-up, those exposed early in life seem to be especially sensitive to the effects of radiation. Evidence for this comes both from the A-bomb survivors and from studies of medically exposed subjects. The main change in risk seems to be over the period of childhood, with less evidence of change after age 20 or so. For this reason, as well as limitations in statistical power, the decrease with age at exposure is not likely to show up in worker studies where subjects are exposed only in adulthood. An additional reason why the effect might not show up in worker studies is that it is strongest for thyroid, breast and non-melanoma skin cancers, none of which are likely to contribute greatly in predominantly male worker mortality studies.

Studies of populations exposed at high doses have not provided evidence of increased sensitivity for those exposed late in life. Both the A-bomb survivor cohort and several of the medically exposed cohorts include subjects exposed at ages over 50. It seems unlikely that an effect large enough to be detected in the relatively low-dose worker studies would not be detected or would be masked by bias at higher doses if it were present.

The age-at-exposure finding based on the worker data is provocative. At least in the analyses of the Oak Ridge National Laboratory data, the investigators have made reasonable efforts to adjust for several related time-dependent variables, and the effect has survived. However, it may not be possible to do this fully given the very strong interrelationships of age at exposure, calendar period of exposure, and birth cohort.

The age-at-exposure effect in the Hanford and Rocketdyne cohorts appears to be dominated by lung cancer (1, 2). Although I agree with Dr. Wing that a very specific type of bias is required to create the age effect that has been found, smoking is a much stronger risk factor for lung cancer than even high-dose exposure to radiation. In addition, smoking habits have changed with time and are known to depend on socioeconomic factors. Given that studies of low-dose effects are highly susceptible to bias, it does not seem unreasonable that bias related to smoking might have contributed to the observed age-at-exposure effect. In addition, the Rocketdyne analyses were not adjusted for calendar year, potentially important since both lung cancer rates and cumulative doses tend to increase with calendar year.

It is important to investigate this effect in other worker cohorts. No age-at-exposure effect was found in the combined international worker study (including workers in the U.S., UK and Canada) even though it included the Hanford and Oak Ridge National Laboratory cohorts (3).

It has been exciting to watch the evolution of statistical methods that have been applied to the A-bomb survivor data. This perhaps started with Dr. Land's work in the 1970s. Then with increased computer capabilities and the maturing of the cohort, the methods being used today have evolved. Drs. Preston and Pierce have been leaders in this effort, although others have contributed. The ERR models have become the standard method of analyzing not only data on A-bomb survivors but on other radiation-exposed cohorts as well. More recently, it is being recognized that EAR models can provide useful alternative descriptions of the data.

I will conclude by thanking our three speakers for stimulating our thinking on age and time effects.

## References

1. B. Ritz, H. Morganstern and J. Moncau, Age at exposure modifies the effects of low-level ionizing radiation on cancer mortality in an occupational cohort. *Epidemiology* **10**, 135–140 (1999).
2. E. S. Gilbert, D. L. Cragle and L. D. Wiggs, Updated analyses of combined mortality data for workers at the Hanford site, Oak Ridge National Laboratory and Rocky Flats Weapons Plant *Radiat. Res.* **136**, 408–421 (1993).
3. E. Cardis, E. S. Gilbert, L. Carpenter, G. Howe, I. Kato, B. K. Armstrong, V. Beral, G. Cowper, A. Douglas and L. D. Wiggs, Effects of low doses and low dose rates of external ionizing radiation: Cancer mortality among nuclear industry workers in three countries. *Radiat. Res.* **142**, 117–132 (1995).

## VI. INTERPRETING CELLULAR AND EPIDEMIOLOGICAL DATA OF PROTRACTED EXPOSURE

Chair: James Smith

*Centers for Disease Control and Prevention, Atlanta, Georgia*

### Dose-Rate Effects and Radiation Protection

R. J. Michael Fry

*Life Sciences Division, Oak Ridge National Laboratory,  
Oak Ridge, Tennessee*

### Introduction

Not without reason and even evidence, it has long been held that the probability of late effects, and importantly cancer, was lower when exposure to radiation was incurred at a low dose rate rather than at a high dose rate. The introduction of a dose-rate factor into risk estimates for radiation protection purposes was made in the 1977 UNSCEAR Report (1). Sir Edward Pochin, a thoughtful but pragmatic scientist, made an estimate of the risk of cancer (solid tumors and leukemias) after exposure to radiation. At that time there were too few data for solid cancers from the atomic bomb survivors to estimate the risk directly, but the majority of leukemias had occurred.

Pochin estimated the risk of mortality from leukemia (all types pooled) based on a linear fit of the dose response. The shape of the dose–response curve suggested a shallower slope at low doses than at the higher doses by about a factor of 2.5. As the interest was in the effect of doses in the range of importance to radiation protection for stochastic effects, he divided the risk estimate based on the linear fit by 2.5. The question then was what would the final ratio of leukemias to solid cancers be, an essential piece in the puzzle of estimating the risk of total cancers. Robin Mole, one of the outstanding experts in radiation carcinogenesis at the time, suggested that the ratio of solid cancers to leukemias would eventually reach 5:1 (the ratio is now predicted to be higher), and so Pochin multiplied the risk for leukemia by 5. When this was done for the data for females and males, the estimate came out at the convenient figure of  $1 \times 10^{-2} \text{ Gy}^{-1}$ . And that was the risk estimate on which protection standards were based until ICRP in their 1990 recommendations (2) used the new estimates from the 1988 UNSCEAR Report (3)!

### DDREF and its Predecessor DREF

In the 1991 ICRP report (2), the term dose-dose-rate-effectiveness factor (DDREF) was introduced. This was an interesting extension of the term dose-rate-effectiveness factor coined by NCRP in 1960 (4). Interesting, because the implication was that the dose–response relationship for cancers was linear-quadratic. NCRP made no such assumption. The DREF was defined as the ratio of the effect per rad at high dose rate and the effect per rad at a low dose rate. The effect per rad was based on the

linear regressions of the data obtained with exposures at high and low dose rates.

#### Variations on the Theme

For many years there have been sporadic reports of increased effectiveness at very low dose rates. The mechanism of such a phenomenon, if true for cancer (most of the reports are for genetic effects), is an enigma unless it represents one aspect of the complex dose response of the effects of radiation on DNA repair. It is not an observation that the devotees of hormesis have taken to heart!

Others have devoted some time to inverse dose-rate effects at somewhat higher doses seen in studies on cells *in vitro*. The same term has, unfortunately, been applied to findings with radon where lower exposure rates have resulted in a higher induction rate of lung cancer than with higher exposure rates. Since the exposures are to  $\alpha$  particles, the term dose rate seems suspect. There are differences in protraction and dose rate. Although low-dose-rate exposures are protracted, there can be different biological factors at play. Consider the simple example that a protracted exposure may be less effective purely because with an exposure that is sufficiently protracted, the age-dependent reduction in susceptibility to induction of cancer may come into play. There are other ways that protraction can affect the behavior of initiated cells, such as through the effects on cytokines and their control. Time is not a simple matter when it comes to biology.

The role of radiation-induced genomic instability has been suggested as central to the induction of cancer by radiation. The suggestion is attractive because it is a possible explanation of how a single exposure to radiation could result in multiple mutations leading to cancer quite some time after the exposure. Most of the current data is for high doses and much of it for high-LET radiations. It will be very important to delineate the role of dose rate in the induction of genomic instability.

#### What is the Future for the DDREF?

The apparent linearity of the dose–response curve for total cancers as a function of dose in the atomic bomb survivors raised the question in some minds that perhaps there was not an effect of dose rate. There is no absolute evidence that a linear dose response up to high doses implies a lack of a dose-rate effect. Perhaps more pertinent is whether the apparent linearity says anything about the dose response of the initial event. It is on this question that Goodhead (5) has written recently. He points out that analysis of FISH-painted chromosomes indicates that chromosome exchanges can be induced by damage due to a single track to only one chromosome and that the response is linear. It is the complex aberrations that contribute the curvature to the responses. Goodhead believes that there is little justification for a DDREF greater than 1 and that the application of the linear-quadratic model to the interpretation of radiation carcinogenesis is, in other words, a snare and a delusion. It is not so easy to claim that dose rate, protraction and fractionation have no significant effect on the induction of cancer.

Since the greatest contribution to the uncertainty of the current estimates of risk of induction by low doses of radiation lies in the choice of the value of the DDREF (6), there is a compelling need to resolve the mysteries of time in relation to how cells, tissues and whole organisms react to radiation.

#### References

1. UNSCEAR, *Sources and Effects of Ionizing Radiation*. No. E77.IX.1, United Nations, New York, 1977.
2. ICRP, *1990 Recommendations of the International Commission on Radiological Protection*. ICRP Publication 60, *Annals of the ICRP*, Vol. 21, Pergamon Press, Elmsford, NY, 1991.
3. UNSCEAR, *Sources, Effects and Risks of Ionizing Radiation*. No. E.88.IX.7, United Nations, New York, 1988.
4. NCRP, *Influence of Dose and Its Distribution in Time on Dose-Response Relationships for Low LET-Radiation*. Report No. 60, Na-

tional Council on Radiation Protection and Measurements, Bethesda, MD, 1980.

5. D. T. Goodhead, Clustered damage to DNA: Time to re-evaluate the paradigm of radiation protection. In *Radiation Research*, Vol. 2 *Proceedings of the Eleventh International Congress of Radiation Research* (M. Moriarty, C. Mothersill, C. Seymour, M. Edington, J. F. Ward and R. J. M. Fry, Eds.), pp. 342–347. Allen Press, Lawrence, KS, 2000.
6. NCRP, *Uncertainties in Fatal Cancer Risk Estimates used in Radiation Protection*. Report No. 126, National Council on Radiation Protection and Measurements, Bethesda, MD, 1997.

### Protraction Effects in Radiation Studies: Basic Biophysics

D. J. Brenner

*Center for Radiological Research, Columbia University,  
New York, New York*

(with R. K. Sachs, University of California, Berkeley)

The dose rates relevant to radiation biophysics cover an enormous range. The exposures of the A-bomb survivors were effectively instantaneous, but environmental or occupational dose rates down to mGy/year are of interest. When a given dose is protracted, various processes can decrease, increase or leave unchanged the biological response. A protracted exposure can be either continuous or in a series of acute fractions; these alternatives may not differ too much if the number of fractions is large. We discuss dose protraction here mainly in terms of splitting a given acute dose  $D$  into two equal fractions  $D/2$  separated by some interval, but the same trend putatively holds, details apart, for any other kind of dose protraction.

#### Protraction and the Acute Dose–Response Curve

With some limitations (see below), one can often consider the effect of protraction in a fairly model-independent way by considering the response to a fractionated exposure as the result of repeating the dose–response relationship for each fraction ( $I$ ). Then, if the acute dose–response relationship has an upward curvature [as in the classic linear-quadratic (LQ) relationship], fractionation would be expected to decrease the response. A decrease of response with increasing dose protraction is often called a *direct* dose-rate effect. On the other hand, downward curvature in the acute dose–response relationship would imply that fractionation increases the response, giving an inverse dose-rate effect. If the acute dose–response relationship is more complex, fractionation could either decrease or increase the response, depending on the dose. On the other hand, a system whose dose–response relationship for acute irradiation is linear—even if linearity resulted from the cancellation of various curved dose–response relationships—would be expected to show little protraction effect.

The applicability of this rule—that the effect of fractionation approximates repeated applications of the same initial part of the dose–response curve—depends on how a cell population changes between dose fractions (or during continuous irradiation). The rule holds if there is restoration of radiosensitivity properties between fractions, so that the distribution of sensitivity within the cell population is the same just before the second fraction as it was before the first fraction. Restoration can occur through repair or other biological processes such as progression of cells in radio-resistant parts of the cell cycle to sensitive parts and *vice versa*. But the rule does not hold if the first dose more or less permanently distorts the cell population structure, e.g. by removing most of a genetically different sensitive subpopulation. The rule can also fail if the first fraction initiates new biological processes, e.g. if induced resistance develops between fractions and persists until the time of the second fraction.

Where the fractionation rule can be tested (i.e. in the laboratory), it often does seem to hold, implying that low-dose response and the effects

of protraction are inextricably linked and, in some sense, represent the same phenomenon.

#### *Dose-Response Relationships with Upward Curvature*

Upwardly curving acute dose-response curves are indeed frequently associated with a direct dose-rate effect (review in ref. 2). A classic mechanism leading to acute dose-response relationships with upward curvature is DNA double-strand break (DSB) repair. For example, for radiation-induced leukemia, the basic model is: (1) leukemias are caused by the induction of chromosomal translocations; (2) translocations in turn require the production of two DSBs; (3) if these two DSBs are produced in a fractionated exposure at different times, the first DSB could be repaired before the second is formed, in which case that DSB pair does not have the potential to make a translocation, as it would if both DSBs were formed at the same time in an acute exposure. This argument would not apply to translocations produced entirely by a single track of radiation, which is probably the dominant mode of translocation formation at very low doses (i.e. when the linear term dominates over the quadratic in the LQ equations), so the doses at which this DSB repair phenomenon tends to dominate are comparatively high.

Most dose-response relationships having (or appearing to have) a threshold can be considered in the present context. That is, protraction would be expected to decrease the response (assuming restoration occurs).

#### *Dose-Response Relationships with Downward Curvature*

While various explanations have been suggested for an initial downward curvature in the acute dose-response relationship at low doses (sometimes referred to as low-dose hypersensitivity), most interpretations involve saturation of damage to a radiosensitive subpopulation of cells. For example, for the end point of oncogenesis, some small subpopulation would be transformed, or stimulated to become less sensitive, even if only a fairly small dose is given. There appears to be some evidence that such a hypersensitive subpopulation may be affected by a damage signal, rather than directly by radiation—a manifestation of the so-called bystander effect. Such a small subpopulation could be genetically or epigenetically different, or it could be in a narrow window of the cell cycle, or perhaps it could be cells temporarily possessing some endogenous, repairable, non-radiative damage. The detailed models are different for these cases, but the resulting acute dose response is rather similar. Protraction could then increase response if the cell population structure is restored on time scales comparable to the protraction time.

#### *Dose-Response Relationships with a Complex Shape*

For X rays, there is evidence, at least *in vitro*, for a complex response: initial downward curvature at low doses, followed by a region of upward curvature (the classic LQ) at somewhat higher doses, followed perhaps by a high-dose plateau. Some *in vitro* oncogenic transformation studies using X rays show such a complex acute dose-response relationship leading to an inverse dose-rate effect at low doses (3), as would be expected on the arguments given above.

#### *Linear Dose-Response Relationships*

Almost all mechanistically based biophysical models predict a linear response with dose at very low doses, though the dose below which linearity occurs is a matter of much debate. As the acute dose is reduced to the point where this linearity dominates, one would not, in the picture outlined above, expect any dose-rate effects, whatever the mechanism. An example can be seen in the analysis of dose-rate effects from radon exposure. Here, at relatively high doses, there is clear evidence of an inverse dose-rate effect; the explanation probably relates to a subpopulation of very radiation-sensitive cells. However, as the radon exposure is reduced to the level where it is very rare for a single cell to be traversed by more than a single particle (and thus a linear dose-response relationship is expected), there are no dose-rate effects of any kind. This phe-

nomenon was first predicted theoretically and then demonstrated epidemiologically—a nice example of synergy between radiation biology and radiation epidemiology.

#### *Conclusions*

Protracting acute exposures can increase, decrease or leave unchanged the biological response. Dose-rate effects can be intimately related to low-dose acute response, and in these situations each can give us clues to the other. Making the assumption that protraction leaves response unchanged could result in either overestimates or underestimates of risk, much as linear extrapolations from high to low doses could result in either overestimates or underestimates. Epidemiological studies and mechanistically based extrapolations probably offer the main hopes for improving low-dose or low-dose-rate risk estimates.

#### *Acknowledgments*

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#### *References*

1. H. H. Rossi, E. J. Hall and M. Zaider, The role of neutrons in transformation research: I. Theory. In *Neutron Carcinogenesis* (J. J. Broerse and G. B. Gerber, Eds.), pp. 371–380. Report EUR 8084, Commission of the European Communities, Luxembourg, 1982.
2. R. K. Sachs, P. Hahnfeldt and D. J. Brenner, The link between low-LET dose-response relations and the underlying kinetics of damage production/repair/misrepair. *Int. J. Radiat. Biol.* **72**, 351–374 (1997).
3. R. C. Miller, E. J. Hall and H. H. Rossi, Oncogenic transformation of mammalian cells *in vitro* with split doses of x rays. *Proc. Natl. Acad. Sci. USA* **76**, 5755–5758 (1979).
4. J. H. Lubin, J. D. Boice, Jr., C. Edling, R. W. Hornung, G. Howe, E. Kunz, R. A. Kusiak, H. I. Morrison, E. P. Radford and S. X. Yao, Radon-exposed underground miners and inverse dose-rate (protraction enhancement) effects. *Health Phys.* **69**, 494–500 (1995).

### **Protraction Effects in Radiation Studies: Epidemiology**

Elaine Ron

*Radiation Epidemiology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland*

Although a great deal is known about the carcinogenic effects of acute or high-dose-rate radiation exposure in humans, much less is known about the effects associated with low-dose-rate and fractionated exposures. As a result, risk estimates are based mainly on populations exposed to radiation delivered at high dose rates. However, protracted exposures over a period of time are more relevant for human experiences. To extrapolate from high to low dose rates, the term “dose and dose-rate effectiveness factor” (DDREF) was introduced by the International Commission on Radiation Protection (ICRP). The DDREF is a factor by which the biological effect caused by a specific dose changes at low compared to high dose rates. Currently the ICRP and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) suggest that compared with risks from acute or high-dose-rate exposure, risks from fractionated or low-dose-rate exposure should be reduced by a factor of 2 or 3, respectively (1, 2).

There is a fairly large body of literature on protraction effects from animal studies. In addition, experiments on cell transformation in culture, somatic cell mutations *in vitro*, and germ cell mutations *in vivo* have added to this literature. These studies have documented that the effects of dose vary depending on the level of protraction. Because findings from animal studies differ depending on species and strain, and cell transfor-

mation and mutation studies are not directly relevant to the carcinogenic process, it is difficult to extrapolate their results to humans (3). Nevertheless, they provide insights into the likely effects of dose rate and fractionation, and have indicated that given the same dose of low-LET radiation, damage is greater at high dose rates than at low dose rates.

It has been suggested that protraction reduces tumorigenic effects because there is an opportunity for cellular repair of sublethal damage, cells redistribute among the phases of the cell cycle, and enhanced cell proliferation compensates for some of the lethal damage. But for high-LET radiation, the influence of protraction is more variable. After a reduction in dose rate, tumor induction can decrease, remain the same, or increase. This may be due to the combination of the high potential for molecular damage, the minimal chance for repair and redistribution, and the potential for compensatory cell proliferation to provide more targets for cell damage. High-LET radiation delivered at low dose rates may also allow exposure to a greater number of cells during the radiosensitive interval ( $G_2$  phase) of the cell cycle. This is because at low dose rates cells often progress through the cycle, but become arrested in the  $G_2$  phase, whereas at high dose rates they immediately stop cycling when irradiated and do not accumulate in  $G_2$  phase (4).

The impact of protraction can be modified by several factors, many of which are highly correlated and dependent on time. In addition to high or low LET, the number of fractions, interval between fractions, duration of exposure, total dose, organ dose, age at exposure, organ sensitivity, concomitant exposures, and reason for radiation exposure all may play important roles, individually or jointly, in determining the consequences of protraction on the induction of cancer. Unfortunately, the data from most studies are too sparse to disentangle these complex relationships.

In reviewing the literature on exposure of humans to low-LET radiation, there is some indication that there may be a dose-rate effect for leukemia and cancers of the lung and thyroid, but there is less support for such an effect for breast cancer (5). For leukemia, there is strong evidence of an excess risk after high-dose-rate exposure at any age. Data from nuclear workers provide some direct information on the health consequences of chronic exposure. While excess risks consistent with the atomic bomb survivor studies have been reported after low-dose-rate exposure, the uncertainties related to these risks are extremely large and the association is weaker. Elevated risks of lung cancer are observed after high-dose-rate radiotherapy and among A-bomb survivors. In contrast, there is little evidence of carcinogenic effects after fractionated or low-dose-rate exposure, even when the cumulative doses to the lung were large. For thyroid cancer, large radiation-associated risks are observed after childhood exposure to the atomic bombs and radiotherapy, but little or no excess risk is seen after adult exposure. After fractionated or low-dose-rate internal or external exposure, most informative studies concern adult exposures. Future quantitative risk estimates from Chernobyl should help to clarify risks to the thyroid gland from protracted radiation.

Unlike the patterns observed for the cancers mentioned above, for breast cancer there is confirmation of a strong radiation effect not only after adult and childhood high-dose-rate exposure (e.g. A-bomb survivors, infants treated for enlarged thymus gland), but also after highly fractionated exposure (e.g. TB fluoroscopy and scoliosis patients). Furthermore, a pooled analysis of A-bomb survivors, patients treated for mastitis, and patients receiving diagnostic fluoroscopies and more recent parallel analyses of A-bomb survivors and fluoroscopy patients demonstrated similar risk estimates even though dose rates differed. However, it has been suggested that a fractionation effect was masked by the difference in the effects of X and  $\gamma$  radiation.

There are few studies on the effects of high-LET radiation in humans, and fewer still include significant numbers of children. Most of our understanding is based on patients treated with radium or Thorotrast, radium dial painters, and uranium miners. Only Thorotrast seems to be leukemogenic. However, Thorotrast was administered in a colloid solution which in itself may be carcinogenic. A statistically significant elevated risk of thyroid cancer was found among a subset of radium dial painters. Based on this single study with only two exposed thyroid cancer cases, it is not possible to draw any conclusion about protraction effects. Sig-

nificant excesses of breast cancer have been observed among British radium dial painters and patients in Germany who were treated with radium. These data are not sufficient for comparing high and low dose rates. A clear dose response for lung cancer is seen among uranium miners who receive highly protracted exposure to radon. Consistent with some experimental data on the effects of high-LET radiation, a pooled analysis of 11 miner cohorts found that lung cancer risk increases as exposure rate decreases. This inverse exposure-rate effect has been observed in most miner studies. An excess risk of lung cancer mortality has also been observed among nuclear workers in Russia who were exposed to plutonium. Further information from this study is needed before direct comparisons can be made with the atomic bomb survivors.

In trying to summarize dose-rate effects in humans, the limitations of the existing data quickly become apparent. Because cumulative doses are generally much smaller in the low-dose-rate cohorts than in the high-dose-rate cohorts, it is difficult to make appropriate comparisons. Often when different medical treatment regimens were employed, e.g. benign gynecological diseases treated with high-dose-rate X rays and low-dose-rate  $^{226}\text{Ra}$ , there was no overlap in cumulative dose, so dose-rate effects cannot be separated from dose effects. In the occupational setting, conducting the large studies required for adequate statistical power, as well as problems involved in estimating doses and other work-related carcinogenic exposures, complicates evaluating dose-rate effects. Environmental studies frequently have not had the statistical power or the methodological attributes needed to detect effects. Human data do not allow drawing firm conclusions about dose-rate effects; however, taken together with the experimental data, a two- to threefold reduction in the carcinogenic effects of fractionated and low-dose-rate exposures, as suggested by ICRP and UNSCEAR, is not unreasonable.

#### References

1. ICRP, *1990 Recommendations of the International Commission on Radiological Protection*. Publication 60, *Annals of the ICRP*, Vol. 21, Pergamon Press, Oxford, 1991.
2. UNSCEAR, *Sources and Effects of Ionizing Radiation*. No. E94.IX.2, United Nations, New York, 1993.
3. R. J. M. Fry, Effects of low doses of radiation. *Health Phys.* **70**, 823-827 (1996).
4. E. J. Hall, *Radiobiology for the Radiologist*, 4th ed. J. B. Lippincott, Philadelphia, 1994.
5. UNSCEAR, *Sources and Effects of Ionizing Radiation*. United Nations, New York, in press.

### Correcting for Exposure Measurement Error in Uranium Miners Studies: Impact on Inverse Dose-Rate Effects

D. O. Stram

*Department of Preventive Medicine, University of Southern California, Los Angeles, California*

(with M. Huberman and B. Langholz)

High-dose-rate short-term exposures are generally subject to larger relative errors in estimation than are lower-dose-rate but longer-term exposures. If the errors in estimating dose rate are large enough relative to the population distribution of true dose rate, this will lead to an artifactual inverse dose-rate effect when estimated exposure is used in survival analysis. We have recently reassessed the data on the Colorado Plateau uranium miners (1) by adopting a multilevel model for measurements of dose rate and then using this model to recompute dose histories using single imputation.

#### Statistical Model for Estimation of Dose Rate

We redid the estimation of dose rate for the Colorado Plateau mines by fitting a multivariate normal distribution to the log of the measured

exposures in WL with a covariance structure that corresponded to a multilevel random slopes and intercepts model exploiting the same hierarchy of state, district and mine used by the Public Health Service (PHS) in assigning the original doses. Dose-rate estimates for all mine-years were derived conditionally upon the measurements as follows.

#### Revised Dose Estimates

We computed our revised dose-rate estimates as

$$\text{Avg}(\text{true dose rate} \mid \text{all measured dose rates}) \quad (1)$$

for all mine-years of interest. The work histories for the individual miners in the study were linked to the revised mine-year dose-rate estimates to construct revised miner exposure histories to use in the analysis.

#### RR Models

The models fitted by Stram *et al.* (1) all use a basic (ERR) model,  $\lambda(t)[1 + \beta X(t)]$ , where  $\lambda(t)$  is the background hazard of lung cancer at age  $t$  and  $X(t)$  is the total exposure of that individual by age  $t$ . This basic dose-response relationship is modified by dose rate in two of the models and by age and time since exposure as well as dose rate in a third. A "mechanistic" dose-rate model was also considered in which the Poisson probability of traversals of a cell by at least one  $\alpha$  particle during some critical part of the cell cycle is incorporated in the model.

For all models, our adjusted analysis reduced the estimate of the magnitude of the inverse dose-rate effects by about 40%. In general, the adjusted exposure estimates gave dose-response relationships which are similar at low doses rates, but considerably higher at high dose rates, compared to the PHS doses.

#### Remaining Statistical Issues

Prentice (4) pointed out that the calculation of revised dose estimates according to Eq. (1) does not fully address the issues of bias in Cox regression analysis. In the case where errors are independent, the revised dose estimates need to be calculated separately at each failure time for all members in each risk set according to

$$\text{Avg}(\text{true dose rate} \mid \text{all measured dose rates and that } T > t-). \quad (2)$$

Here  $T$  is survival time (age) and  $t$  is the age at death which defines the group at risk (risk set). The fact that all subjects in the risk set survived to at least age  $t-$  is informative about the distribution of true exposure for the members of the risk set, and this information may need to be taken into account in adjusting exposure.

*Shared dose errors.* An additional complicating factor in the Colorado Plateau cohort is that miners who worked in the same mine-years share the same dose errors. The technically correct adjustment to dose is to include in the conditioning in Eq. (2) the survival times up to the calendar time of the index death for all miners who had worked in the same mine-years as any member of the risk set. The importance of our neglect of this issue in the Colorado analysis is now being addressed.

#### Monte Carlo Maximum Likelihood Methods

Since the above adjustments for dose rate needed in the Cox model are very complex, we have adopted a simulation approach to estimating the impact that the neglected issues of shared dose errors and temporal changes in  $\text{Avg}(\text{true dose rate})$  may have on analyses of the Colorado Plateau cohort. Comparing parameter estimates and the lengths of corresponding confidence intervals based upon Monte Carlo maximization to those obtained using the adjusted doses as described above will elucidate the extent to which the issues of shared dose errors and temporal changes in the dose distribution have distorted inference in previous analyses of the Colorado Plateau uranium miner cohort.

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#### References

1. D. O. Stram, B. Langholz, M. Huberman and D. C. Thomas, Correcting for exposure measurement error in a reanalysis of lung cancer mortality for the Colorado Plateau uranium miners cohort. *Health Phys.* **77**, 265–275 (1999).
2. National Research Council, Committee on the Biological Effects of Ionizing Radiation, *Health Effects of Exposure to Radon (BEIR VI)*. National Academy Press, Washington, DC, 1998.
3. F. Lundin, J. Wagoner and V. Archer, *Radon Daughter Exposure and Respiratory Cancer: Quantitative and Temporal Aspects*. Report from the Epidemiological Study of United States Uranium Miners, 1971.
4. R. L. Prentice, Covariate measurement errors and parameter estimation in a failure time regression model. *Biometrika* **69**, 331–342 (1982).

## DISCUSSION

Jerome Puskin

*U.S. Environmental Protection Agency, Potomac, Maryland*

From a radiation protection policy standpoint, the most important—and most controversial—issue is the extrapolation of radiogenic cancer risk estimates, based on modeling of epidemiological data, to the low-dose-rate conditions most relevant to environmental and occupational exposures.

In the case of low-LET radiation, the linear-quadratic (LQ) model has provided a widely accepted framework for this extrapolation. For acutely delivered radiation, the LQ response increases proportionally to dose at low doses but curves upward at higher doses. The magnitude of the response for low dose rates (or for highly fractionated doses) is predicted to be the same as the slope of the low-dose component of the acute dose-response curve. Thus, in cases where a linear dose response is observed in cohorts exposed to acute radiation, about the same risk per unit dose is predicted at low dose rates.

This hypothesis can be tested through a comparison between results obtained for acutely exposed A-bomb survivors and for U.S. and Canadian fluoroscopy patients, who received highly fractionated doses to the breast and lung. In the A-bomb survivors, the observed dose response is approximately linear for both lung and breast cancer. In agreement with the model, the risk per unit dose for breast cancer in the fluoroscopy patients is about the same as in the A-bomb survivors (1, 2). However, no evidence of a dose response for lung cancer is seen in the fluoroscopy patients. The upper bound on the ERR/Sv appears to be nearly an order of magnitude lower than the best estimate of lung cancer risk derived from the RERF data (3).

This discrepancy in findings with respect to lung cancer suggests that the LQ model may be an unreliable method for estimating the risk at low dose rates. If so, the RERF data may be of little value in estimating risks from environmental exposures. Several factors, however, might help to resolve the discrepancy within the context of the model. First, errors in the RERF dosimetry could mask upward curvature in the dose-response relationship for lung cancer (4). Second, the baseline incidence of lung cancer is several times lower in the U.S./Canadian population compared to the Japanese A-bomb survivor population. Consequently, if the absolute risk of radiogenic lung cancer is transportable across populations, the ERR/Sv would be substantially reduced from what has been estimated in the RERF study. Finally, confounding by the disease status of the fluoroscopy patients, who were being treated for tuberculosis, could also mask an increase in lung cancer incidence with radiation dose. For ex-

ample, patients who had more intractable cases of TB may tend to have had higher doses but be more likely to have given up smoking.

In the case of high-LET radiation doses from inhaled radon progeny, epidemiological data on underground miners indicate an inverse exposure-rate effect, with increasing risk as the exposure becomes more protracted. As we have heard, the inverse exposure-rate effect can arise from a sensitive window in the cell cycle for transformation or, alternatively, from longer-term promotional effects of the radiation. Based on the former mechanism, and supporting data on radon-induced lung cancer in rats, the risk should reach a plateau once the exposure rate reaches a critical minimum level. It is unclear whether or not this point was reached in the epidemiological studies, so it is possible that the risk at typical residential exposure rates could be even higher than inferred from the miner studies. On the other hand, some biologically based models of carcinogenesis may project lower risks or even a possible protective effect of radiation at residential exposure rates. Resolution of this question is most likely to come from case-control studies of the correlation between lung cancer incidence and residential radon exposure. To date the results of such studies tend to support the reasonableness of current risk estimates.

#### References

1. G. R. Howe and J. McLaughlin, Breast cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy study and a comparison with breast cancer mortality in the atomic bomb survivors. *Radiat. Res.* **145**, 694–707 (1996).
2. M. P. Little and J. D. Boice, Jr., Comparison of breast cancer incidence in the Massachusetts tuberculosis fluoroscopy cohort and in the Japanese atomic bomb survivors. *Radiat. Res.* **151**, 218–224 (1999).
3. G. R. Howe, Lung cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy study and a comparison with lung cancer mortality in the atomic bomb survivors. *Radiat. Res.* **142**, 295–304 (1995).
4. D. A. Pierce and M. Vaeth, The shape of the cancer mortality dose-response curve for the A-bomb survivors. *Radiat. Res.* **126**, 36–42 (1991).

## VII. KEYNOTE ADDRESS

Chair: Jay Lubin

*National Cancer Institute, Bethesda, Maryland*

### Major Cancer Susceptibility Genes and Radiation: What Do We Know?

Margaret Tucker

*National Cancer Institute, Bethesda, Maryland*

Over 30 major susceptibility genes for cancer have been described in recent years, but few have been studied in population settings. With the major susceptibility genes, mutations or variations are infrequent. A large number of genes which are important in DNA repair or the metabolism of carcinogens and have relatively frequent variations (polymorphisms) have also been identified and discussed earlier in the meeting. Studying any of these genes is complicated; study designs have all of the difficulties of traditional epidemiological designs, with the additional complexity of laboratory components. The identification of these genes has been invaluable for beginning to understand some of the mechanisms of carcinogenesis, but for many of the genes, appropriate human studies have not been completed (1).

One of the best studied of these genes is *RBI*, mutations in which

cause heritable retinoblastoma (2, 3). *RBI* is a large, complex gene that is difficult to fully sequence. There are no known “hotspots”, so there are no easy methods to characterize mutations. No large studies of genotype and phenotype correlations have been possible to date because of the technical difficulties in mutation analysis. For population studies, the phenotype of heritable retinoblastoma, which is either bilateral or unilateral familial, has been used as a surrogate for probable *RBI* mutations. Individuals with heritable retinoblastoma are at risk of ionizing radiation-related cancers, with a dose response for both bone and soft tissue sarcomas (4, 5). The information that is available is based on therapeutic-range doses from radiation therapy for retinoblastoma. Dose for dose, the risk of bone sarcoma after retinoblastoma appears similar to that of bone sarcoma after other childhood malignancies (4). The radiation-related sarcomas also appear earlier than the “spontaneous” bone sarcomas in limbs, within 5 years of the irradiation. Less well known is that individuals with heritable retinoblastoma are also at increased risk of melanoma (5, 6). Individuals with retinoblastoma and melanoma often have dysplastic nevi, a precursor lesion for melanoma. The risk of melanoma is likely related to UV radiation, but this has not been quantified. Although speculative, it seems likely that as the cohort of retinoblastoma survivors ages, they may be at increased risk of additional adult-onset cancers, perhaps related to other exposures.

Nevoid basal cell carcinoma syndrome (NBCCS) is a highly penetrant autosomal dominant trait with variable expressivity and a distinctive phenotype (6). Clinical features of the phenotype include multiple basal cell carcinomas, palmar and plantar pits, odontogenic keratocysts, medulloblastoma, ovarian fibromas, and ectopic calcifications. *PTCH*, the major gene for NBCCS, is large, again with no specific “hotspots” that would simplify mutation testing. *PTCH* acts as a tumor suppressor in NBCCS and plays a critical role in normal development as part of the hedgehog signaling pathway. Individuals with NBCCS are at increased risk of skin cancers induced by ionizing radiation and UV radiation (7). Within radiation ports for medulloblastoma, affected individuals develop hundreds or thousands of basal cells. Among affected individuals without medulloblastoma, the basal cells are more frequent in sun-exposed areas (8). Basal cell carcinomas are a less frequent manifestation of NBCCS among African-Americans than among whites (9). Darker skin pigmentation seems to protect against the UV-radiation-induced skin cancers but not those induced by ionizing radiation. No quantitative data exist for either ionizing or UV-radiation dose and risk of skin cancers in NBCCS.

Ataxia telangiectasia is an autosomal recessive trait with affected individuals having immune dysfunction and progressive neurological degeneration. Few live until middle age. Approximately one-third of those affected develop lymphatic malignancies, either lymphoma or leukemia (10). Early on, when radiation therapy was used to treat the lymphomas, clinicians quickly discovered that affected individuals with ataxia telangiectasia have acute radiation sensitivity. Ataxia telangiectasia has thus become a model for radiation toxicity, but no quantitative data exist for humans. Much of the investigation of radiation toxicity has come from cell lines and mouse models, for obvious reasons. Ataxia telangiectasia is caused by mutations in a very large gene, *ATM*. The functions of *ATM* are still being unraveled, but it appears to be important in DNA damage recognition and in cell cycle control. It has been hypothesized that heterozygotes with mutations in *ATM* are at increased risk of radiation-related cancers. Since the cloning of *ATM*, several investigations have tried to directly assess risk of breast cancer and radiation-related cancers in heterozygotes (10, 11). Breast cancer risk is under active investigation; Gatti has recently proposed that truncating mutations are related to disease and missense mutations are related to cancer risk (12). Mutations have not been identified in individuals developing contralateral breast cancer after conservative surgery and radiation (13) or in individuals with second radiation-related cancers after Hodgkin's disease (11).

Mutations in *BRCA1* and *BRCA2* confer increased risk of breast, ovarian and possibly prostate cancer (14). They both are large complex genes, but commercial testing is readily available to identify mutations. In addition, founder mutations have been described in a number of different populations. Unlike some of the other conditions discussed above, breast

cancer related to mutations in *BRCA1/2* does not have a clear phenotype to use as a surrogate for mutation testing. These genes are of interest with respect to radiation because of the relationship of *ATM*, *RAD51* and *BRCA1* (and perhaps *BRCA2*) (10) as well as the known relationship between ionizing radiation at a young age and breast cancer risk. Few data address the question of radiation effects in individuals with *BRCA1/2* mutations. Clinical trial data, however, might be a good resource for evaluating both acute and chronic radiation effects in women treated with conservative surgery and radiation for breast cancer. However, it will take very large numbers, given the rarity of mutations.

The potential for studying the interaction of mutation in these genes (and others) and both ionizing and ultraviolet radiation is great, but it will require close attention to methodological problems. At this time, identification of mutation in these major susceptibility genes in the general population is quite expensive, both at an individual level and especially at the population level even though the mutations are so infrequent. It will take studies with very large numbers of individuals to start to evaluate the gene-environment interactions with radiation. Only very limited data are available to begin to examine gene-radiation dose effects; essentially no quantitative data are available for UV radiation. In the next few years, however, when mutation detection becomes more feasible, these studies should be very informative about the mechanisms of carcinogenesis.

#### References

1. M. A. Tucker. Epidemiologic methods. In *Cancer: Principles and Practice of Oncology*, 6th ed. (V. T. Devita, Jr., S. Hellman and S. A. Rosenberg, Eds.). Lippincott Williams & Wilkins, Philadelphia, in press.
2. D. R. Lohman, B. Brandt, W. Hopping, E. Passarge and B. Horsthemke, The spectrum of RB1 germ-line mutations in hereditary retinoblastoma. *Am. J. Hum. Genet.* **58**, 940-949 (1996).
3. B. L. Gallie, Predictive testing for retinoblastoma comes of age. *Am. J. Hum. Genet.* **61**, 279-281 (1997).
4. M. A. Tucker, G. J. D'Angio, J. D. Boice, Jr., L. C. Strong, F. P. Li, M. Stovall, B. J. Stone, D. M. Greene, F. Lombardi and J. F. Fraumeni, Jr., Bone sarcomas linked to radiotherapy and chemotherapy in children. *N. Engl. J. Med.* **317**, 588-593 (1987).
5. F. L. Wong, J. D. Boice, Jr., D. H. Abramson, R. E. Tarone, R. A. Kleinerman, M. Stovall, M. B. Goldman, J. M. Seddon, N. Tarbell and F. P. Li, Cancer incidence after retinoblastoma: radiation dose and sarcoma risk. *J. Am. Med. Assoc.* **278**, 1262-1267 (1997).
6. M. R. Gailani and A. E. Bale, Developmental genes and cancer: Role of patched in basal cell carcinoma of the skin. *J. Natl. Cancer Inst.* **89**, 1103-1109 (1997).
7. V. E. Kimonis, A. M. Goldstein, B. Pastakia, M. L. Yand, R. Kase, J. J. DiGiovanna, A. E. Bale and S. J. Bale, Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. *Am. J. Med. Genet.* **69**, 299-308 (1997).
8. A. M. Goldstein, S. J. Bale, G. L. Peck and J. J. DiGiovanna, Sun exposure and basal cell carcinomas in the nevoid basal cell carcinoma syndrome. *J. Am. Acad. Dermatol.* **29**, 34-41 (1993).
9. J. F. Korczak, J. S. Brahim, J. J. DiGiovanna, R. G. Kase, L. H. Wexler and A. M. Goldstein, Nevoid basal cell carcinoma syndrome with medulloblastoma in an African-American child: a rare case illustrating gene-environment interaction. *Am. J. Med. Genet.* **69**, 309-314 (1997).
10. K. K. Khanna, Cancer risk and the ATM gene: A continuing debate. *J. Natl. Cancer Inst.* **92**, 795-802 (2000).
11. K. E. Nichols, S. Levitz, K. E. Shannon, D. C. Wahrer, D. W. Bell, G. Chang, S. Hegde, D. Neuberg, T. Shafman and L. Diller, Heterozygous germline ATM mutations do not contribute to radiation-associated malignancies after Hodgkin's disease. *J. Clin. Oncol.* **17**, 1259 (1999).
12. R. A. Gatti, A. Tward and P. Concannon, Cancer risk in ATM heterozygotes: A model of phenotypic and mechanistic differences between missense and truncating mutations. *Mol. Genet. Metab.* **68**, 419-423 (1999).
13. T. D. Shafman, S. Levitz, A. J. Nixon, L. A. Gibrans, K. E. Nichols, D. W. Bell, C. Ishioka, K. J. Isselbacher, R. Gelman and D. A. Haber, Prevalence of germline truncating mutations in ATM in women with a second breast cancer after radiation therapy for a contralateral tumor. *Genes Chromosomes Cancer* **27**, 124-129 (2000).
14. J. P. Struewing, P. Hartge, S. Wacholder, S. M. Baker, M. Berlin, M. McAdams, M. M. Timmerman, L. C. Brody and M. A. Tucker, The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *N. Engl. J. Med.* **336**, 1401-1408 (1997).