Active Research Protocols by Study Program

1 April 2009–31 March 2010

The 95 research protocols (RPs) that were active during the fiscal year are listed below by study program with brief progress reports prepared by primary investigating departments and listings of publications and oral presentations emanating from related studies follow.

Investigating departments are identified by the following codes:

Clinical Studies, Hiroshima **(CH)**
Clinical Studies, Nagasaki **(CN)**
Epidemiology, Hiroshima **(EH)**
Epidemiology, Nagasaki **(EN)**
Genetics **(G)**
Radiobiology/Molecular Epidemiology **(R)**
Statistics **(S)**
Information Technology **(IT)**
RERF Director **(D)**
RERF Assistant Chief of Research **(ACR)**
RERF Chief Scientist **(CS)**

Outside researchers are not listed with their affiliations here.

The following marks are used for publications and oral presentations based on RPs:

♦ Publications
⌘ Manuscripts in Press
❖ Oral Presentations

Protocols are presented by study program in reverse chronological order, and entries include the protocol title, investigators, and a brief description of the RP.

Each study program is followed by a listing of any publications that emanated from these studies as well as manuscripts accepted for publication during the fiscal year. These are presented in alphabetical order by first author. (*RERF Reports* are listed with abstracts before other journal publications.)

Most of the outside authors are RERF consultants, expert advisors, or part-time professionals and their listings with affiliations appear in a separate section of the annual report.

(Japanese) indicates that the original publication is in Japanese.

Oral presentations are included by study program after publications and manuscripts in press, and listed chronologically by meeting date.

Research Protocols 3-08, 2-08, 2-06, 1-75 (Platform Protocol), 2-61, A1-09, A11-08, A9-08, A7-08, A6-08, A3-08, A1-08, A1-07

Life Span Study (LSS)

RP 3-08 Mortality in relation to smoking and other lifestyle factors in a Japanese population

Sakata R (EH), McGale P, Darby S, Grant EJ (EH), Boreham J, Sugiyama H (EH), Soda M (EN), Shimizu Y (EH), Tatsukawa Y (CH), Yamada M (CH), Moriwaki H (EH), Kasagi F (EH), Suyama A (EN), Geyer S (S), Kodama K (CS), Peto R

The question has been raised as to why the magnitude of effects from smoking cigarettes appears to be less in Japan than in western countries. An initial issue is to determine if the magnitude is, in fact, less once identical methods of data definition, stratification, and analysis are used in western and Japanese populations. The LSS provides a unique opportunity to examine this, because of the large cohort size, smoking data gathered repeatedly over >35 years, and complete mortality ascertainment. So a comparison with the extensive British doctors' smoking study (Doll et al., *British Medical Journal* 2004; 328:1519) is being conducted in collaboration with Oxford University. The main analyses focus on individuals for whom there is LSS information on cigarette smoking from the mail surveys. Mortality is being examined in relation to cigarette smoking status, categorized by the same rules used in the analysis in the British doctors' study.

Calculations of relative risks (RR) based on the age- and calendar year-standardized mortality rates (SMR) by categories of smoking status were completed. RRs of current smokers for lung cancer death show comparable levels to those shown in previous studies of Japanese populations (Male, RR = 6, Female, RR = 4). RRs were also calculated

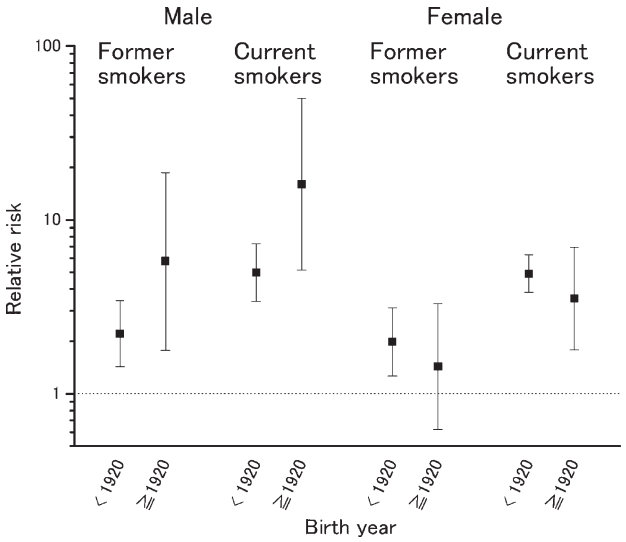


Figure. Relative risk and 95% confidence intervals of current and former smokers to lifelong non-smokers for lung cancer mortality by birth cohort in the LSS subjects.

based on SMR by birth cohort divided into two groups: one with subjects who were born in 1920 or later, and one before 1920. High RRs for all cause and lung cancer mortality are indicated among subjects who were born in 1920 or later, compared with subjects born before 1920 for both males and females (RRs of current smokers for lung cancer death: Male, <1920 RR = 5, 1920+ RR = 16; Female, <1920 RR = 0.9, 1920+ RR = 3.7) (Figure). A series of multiplicative Poisson regression analyses with adjustments made for radiation dose and other variables will be completed in early 2010 and a manuscript will be prepared.

RP 2-08 Mail Survey 2008 on epidemiological factors in the Extended Life Span Study population

Sakata R (EH), Nagano J, Grant EJ (EH), Sugiyama H (EH), Hsu WL (S), Kasagi F (EH), Fujiwara S (CH), Akahoshi M (CN), Moriwaki H (EH), Mabuchi K, Ron E, Suyama A (EN), Ozasa K (EH), Kodama K (CS)

A mail survey has been started on all 47,000 subjects who are alive in the Extended Life Span Study cohort (LSS-E85) in order to update information on epidemiological factors such as lifestyles, history of diagnostic and therapeutic radiation exposure, height, weight, financial situation, disease history, menstruation, and psychosocial factors. Such information will be used to examine factors that may confound or modify the health effects of radiation. Due to the large size, long-term follow-up, and advanced age of the cohort, an effort was made to broaden the focus to include overall aging markers. Additional targeted data include history of major diseases as well as mental and physical health status. A pilot study assuring the validity and reliability of the questionnaire was completed.

To support the approved Research Proposal entitled "Clinical health study for expanded group of younger A-bomb survivors" (RP 3-07), applicants for the clinical health study are being recruited as part of the mail survey to increase the number of participants of the Adult Health Study who were under 10 years of age at the time of bombings; this represents a major collaboration between the Departments of Epidemiology and Clinical Studies. The first phase of the study targets 5,202 persons eligible for recruitment into the Adult Health Study and is nearly completed. The response rate of the first phase was 64% among the total subjects, and it was 84% when the subjects whose questionnaire were returned as undeliverable and those who were found to be dead by returned mails were excluded from the denominator.

Next, a pilot study will be conducted to test the feasibility of collecting doing saliva samples for extracting subjects' DNA. This proposal has received final approval and is in the final planning stages.

RP 2-06 Relationship between radiation exposure and risk of second primary cancers among A-bomb survivors

Li CI, Nishi N (EH), Furukawa K (S), Sugiyama H (EH), Soda M (EN), Sakata R (EH), Hayashi M (EH), Kasagi F (EH), Suyama A (EN), Mabuchi K, Davis S, Kopecky KJ, Kodama K (CS), Ozasa (EH)

The primary goal of this study is to evaluate the relationship between radiation exposure and risk of second primary cancers among LSS A-bomb survivors. In addition to evaluating the risk of second primary cancers by radiation dose, analyses will also be stratified by cancer type, treatments for the first cancer, gender, age at exposure, age at first cancer diagnosis, time since radiation exposure, and time between first and second primary cancers. This study will provide greater insight into the effects of radiation exposure on cancer risk.

The study is conducted under the Radiation Research Partnership Program between the University of Washington and RERF. Drs. Li and Nishi have prepared manuscripts on the risk of second primary cancers by radiation dose using different statistical methods, respectively. Dr. Li reported a similar linear dose-response relationship between radiation exposure and risk of both first and second primary solid tumors from the data through 2002, i.e., ERR/Gy = 0.65, 95% confidence interval (CI): 0.57–0.74 and ERR/Gy = 0.56, 95% CI: 0.33–0.80, respectively. Thus the ERR for second primary cancers was not greater than that for first primary cancers. However, because the baseline risk per person-year is higher for second primary cancer than for first cancer, the excess absolute risk per person-year Gy was higher for second primary cancers than for first cancers. Dr. Nishi confirmed the similarity of the dose-response relations for first and second primary cancers, using observed to expected ratios. He also found that the risk for second primary cancers was higher among those who were younger at the time of bombings and younger at their first cancer diagnosis. The paper by Dr. Li has been submitted to an international journal and the paper by Dr. Nishi is under internal review.

RP 1-75 Research plan for RERF studies of life span of A-bomb survivors, Hiroshima and Nagasaki

Ozasa K (EH), Kodama K (CS), Shimizu Y (EH), Kasagi F (EH), Grant EJ (EH), Sugiyama H (EH), Sakata R (EH), Soda M (EN), Suyama A (EN), Cologne JB (S)

This is the long-term follow-up of a fixed cohort of 93,000 atomic-bomb survivors and 27,000 unexposed individuals. The follow-up began in 1950 using the family registration system, the *koseki*, which assures virtually complete mortality ascertainment. Through tumor registries in Hiroshima and Nagasaki, it has also become possible to study cancer incidence in a large fraction of the LSS. It also serves as the sampling frame for the Adult Health Study clinical subcohort.

Analyses of updated cancer and non-cancer mortality data (through 2003) using the DS02 have been completed. The risk of total causes of death significantly increased, largely because of an approximately linear dose-dependent increase in cancer, especially among subjects who were young at the time of bombing. The linear model provides the best fit over the full-dose range for total solid cancers, with an estimated threshold dose of 0.0 Gy and an upper 95% confidence limit of 0.15 Gy (Figure). The lowest dose range with a statistically significant trend is 0–0.20 Gy (ERR/Gy = 0.56). The risk of cancers significantly increased for most major sites including esophagus, stomach, colon, liver, gall bladder, lung, female breast, ovary, and urinary tract. Although most of the excess leukemia occurred in the early

years after exposure, a small excess leukemia risk has continued even 50 years after radiation exposure. An increased risk of non-neoplastic diseases including circulatory, respiratory, and digestive system was observed at moderate dose or higher, but the degree to which these are causal relationships requires further investigation. There was no evidence of a radiation effect on infectious or external causes of death.

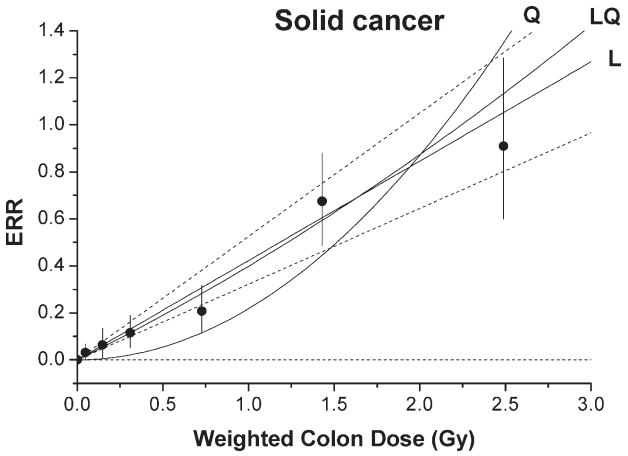


Figure. Dose dependency of excess relative risk (ERR) for solid cancer mortality, showing the linear (L), linear quadratic (LQ), and quadratic (Q) functions. The area between dotted lines is the 95% confidence region for the fitted linear line. Closed circles indicate point estimates of the risk of specific dose category and vertical lines are their 95% confidence intervals.

Detailed mortality analyses have been published for leukemia [RP-A6-08], malignant lymphoma [RP-A1-07], and circulatory diseases (Shimizu et al., *British Medical Journal* 2010; 340:b5349). There is an elevated risk of both stroke and heart disease at moderate doses (>0.5 Gy), but the degree of risk at lower doses is unclear at this time. Prospective data on smoking, alcohol intake, education, occupation, obesity, and diabetes had little impact on the radiation risk estimates for either stroke or heart disease. Analyses for other noncancer diseases such as respiratory disease are underway. The future projection of excess deaths due to radiation exposure and lifetime risk measures (risk of exposure-associated deaths and loss of life expectancy) associated with cancer and noncancer disease deaths were estimated using models selected by a predictive-performance criterion (Furukawa et al, *Risk Analysis* 2009; 29:885–99). At the end of 2005, 40% of LSS subjects, including 86% of those exposed before age 10, were alive, so continued follow-up is essential. The LSS mortality data have demonstrated an excess risk of death from cancers, but the causal nature of associations between radiation exposure and circulatory, digestive, and respiratory diseases is less certain.

RP 2-61 Study of mortality in children exposed *in utero*

Kasagi F (EH), Shimizu Y (EH), Preston DL, Nishi N (EH), Suyama A (EN), Cologne JB (S), Ozasa K (EH), Kodama K (CS)

This is an ongoing long-term follow-up of a cohort of about 3,600 persons who were *in utero* at the time of the atomic bombings. The *in utero* cohort, although small in size, can provide much information on the health effects associated with radiation exposure occurring during embryonic and fetal development. It is the only available cohort in the world with exclusively *in utero* radiation exposure and adulthood data on health risks.

A recently published report (Preston et al., *Journal of the National Cancer Institute* 2008; 100:428–36) showed that the excess risk of adulthood cancer incidence following *in utero* exposure appears to be somewhat smaller than that seen in those exposed as children, and that the temporal pattern of the excess risks following *in utero* exposures also differs from that seen for childhood exposures. In particular, while the excess rate tends to increase markedly with age for those exposed as children, the excess rate does not increase with age for those exposed *in utero*. Relative risks in the earliest years of follow-up (13–20 years after exposure), are reasonably consistent with what one might expect based on other studies of childhood cancer following *in utero* exposure; however there is only weak evidence of an increase in cancer incidence rates for adult-onset cancers.

Analysis of the mortality data for 1950–2003 has been conducted with a special emphasis on temporal patterns of radiation risks for cancer and non-cancer deaths. The preliminary analyses indicated that the sex-averaged ERR of solid cancer mortality at attained age 50 yrs among *in utero* exposure was lower than that among young childhood exposure (less than 6 years of age at time of bombing), in which the temporal pattern significantly differed between *in utero* exposure and that in childhood although the time-averaged ERR did not differ between them. The pattern seen for solid cancer mortality is similar to that reported for the solid cancer incidence data. On the contrary, the sex-averaged ERR of non-cancer mortality at attained age 50 yrs is larger than that observed among those exposed at young childhood; in this case the temporal patterns for the *in utero* and childhood exposure groups did not differ, but the time-averaged ERR suggestively differed between them (p = 0.06) (Figure).

As the potential number of excess cancer cases will increase appreciably in the next few decades, so it is important to continue the follow-up using both mortality and cancer incidence as endpoints.

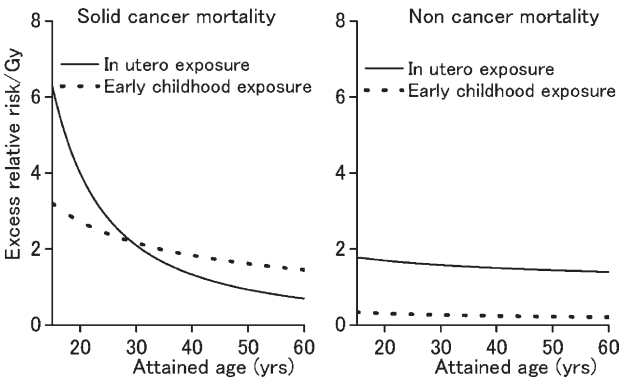


Figure. Mortality risk of cancer and non-cancer diseases in *in utero* cohort compared to LSS subjects exposed in early childhood.

RP-A1-09 Biologically based mechanistic modeling of leukemia in the Life Span Study

Dekkers F, Bijwaard H, Hsu WL (S), Cullings HM (S), Soda M (EN), Sugiyama H (EH), Kasagi F (EH), Suyama A (EN)

At RERF ample experience exists with modeling predominantly solid cancer in the Life Span Study (LSS) whereas the Dutch National Institute of Public Health and Environmental Protection (RIVM) has a history of developing and applying a Moolgavkar-type two-mutation model to leukemia in both laboratory animals and humans. These complementary backgrounds provide a unique opportunity to model the partly radiation-induced leukemia incidence in the LSS in a collaborative effort. Such a biologically-based leukemia model would not only make the comparison of calculated risks for the A-bomb survivors with epidemiological estimates possible, but more importantly: it would provide a means to transfer risks, for instance, to chronic and low dose exposures and to otherwestern populations. Such risk estimates are of great interest for radiation protection purposes. Collaborative and data sharing agreements were completed and statistical analysis is in progress. During a visit to RERF in November 2009 Dr. Dekkers created data files that can be used as input for the two-mutation carcinogenesis (TMC) model from the LSS data on leukemia incidence. Preliminary analyses indicate that the data can be described using the TMC model.

RP-A11-08 Relationship between radiation exposure and kidney disease among A-bomb survivors

Adams MJ, Grant EJ (EH), Kodama K (CS), Shimizu Y (EH), Kasagi F (EH), Suyama A (EN), Sakata R (EH), Fujiwara S (CH), Akahoshi M (CN)

The purpose of this study is to evaluate whether increasing radiation dose is associated with kidney disease mortality in atomic-bomb survivors after adjusting for other known risk factors for kidney disease incidence that were collected among LSS cohort members.

The specific aims are:

Aim 1: Evaluate whether radiation dose is associated with kidney disease mortality after adjusting for known risk factors for kidney disease (age, diabetes mellitus, and hypertension) in the LSS cohort.

Aim 2: Evaluate the prevalence of kidney disease and heart disease as primary/secondary causes of death in atomic-bomb survivors and analyze the association of such events with radiation dose.

Aim 3: Evaluate the validity of self-reports of hypertension and diabetes in the LSS by comparing self-report and actual clinical findings in those individuals who answered a mailed LSS survey and who also participated in the AHS.

Kidney disease-related deaths were classified from death certificate records. Due to possible misclassification issues, categories included "definite," "probable," and "possible." Models of outcome rates were developed that included radiation, BMI, self-reported hypertension, and diabetes status, among others.

Dr. Adams visited RERF as a Beebe fellow between June and September of 2008 during which data were coded and data analyses commenced. At the end of this period, data-sharing agreements were approved so that he can complete his analyses. The results showed a positive dose response for "possible chronic kidney disease" cases with a linear or linear quadratic dose response. Dr. Adam's manuscript will be submitted in 2010.

RP-A9-08 Timing of menarche and first birth in relation to risk of breast cancer in A-bomb survivors

McDougall J, Sakata R (EH), Sugiyama H (EH), Grant EJ (EH), Davis S, Soda M (EN), Shimizu Y (EH), Tatsukawa Y (CH), Kasagi F (EH), Suyama A (EN), Kopecky K, Li CI

This RP was developed under the auspices of the RERF Radiation Research Partnership Program. Ms. McDougall is a doctoral student at the University of Washington. The primary aim of this study is to evaluate the impact of radiation on breast cancer risk among women who had experienced menarche, but had not yet borne a child at the time of the atomic bombings. Our hypothesis is that women who were exposed to radiation from the atomic bombs after reaching menarche but before a first birth will have a higher risk of breast cancer than women who were exposed to radiation before menarche or after their first birth because of the combination of pre-parous breast tissue susceptibility and hormonal stimulation.

The project will employ a cohort design using all women in the LSS who completed at least one lifestyle questionnaire (LSS69, LSS78, or LSS91) or an AHS questionnaire. The primary variables of interest are age at menarche, age at first birth, and radiation dose. Variables that will be of use in assessing confounding include body mass index, cancer type, height, hormone receptor status, and parity. The primary outcome of interest is the first diagnosis of breast cancer, as recorded in the tumor registries of Hiroshima and Nagasaki from 1958 to 2002. An analysis of the timing of reproductive events and breast cancer risk involves comparing radiation dose responses between three groups. (1) Pre-menarche vs (2) menarche to first birth vs (3) after first birth.

Of approximately 30,000 eligible women identified for this study, 9,000 were pre-menarcheal at the time of the bombing, 7,000 were between menarche and first birth, and 14,000 were post first birth. The overall ERR/Gy for all women was 1.55 and the EAR was 12.6/10,000 PYGy. When models for the baseline risk of breast cancer did not include reproductive status ATB, we observed significant dose effect modification by reproductive status ATB. However, after accounting for the significant heterogeneity in the baseline risk of breast cancer between different reproductive-status ATB groups, we did not find evidence of significant dose effect modification. A manuscript has been accepted for publication in *Cancer Epidemiology, Biomarkers & Prevention*.

RP-A7-08 Risk estimates of bladder, ureter and renal pelvis cancers among atomic bomb survivors after adjustments for lifestyle factors

Grant EJ (EH), Kasagi F (EH), Suyama A (EN), Shimizu Y (EH), Soda M (EN), Sugiyama H (EH), Sakata R (EH), Yamada M (CH), Hsu WL (S), Nagano J, De Roos A, Kopecky K, Davis S

The primary goal of this study is to re-evaluate the

radiation risk of urothelial cancers (UC) after accounting for lifestyle factors also known to be associated with these cancers using a stratified case-cohort design. Lifestyle factors to be incorporated in the analyses include smoking, occupational exposures to aromatic amines and polycyclic hydrocarbons, diet, alcohol consumption, and an index for socioeconomic status. This study will help expand our understanding of the radiation risks of cancer in organs that are also susceptible to lifestyle risk factors.

About 4,000 questionnaires, which included handwritten information on a participant's current occupation and industry, are being re-abstracted in an effort to assign probabilities of relevant exposures using a job-exposure matrix. Lifestyle data, including smoking, education, and alcohol consumption, from these questionnaires have already been entered into RERF research databases. These data are being combined across the multiple questionnaires and incorporated into radiation risk models, and a manuscript is planned.

Another manuscript analyzing the association of UC and radiation exposure along with non-occupational lifestyle factors is also being prepared. This full cohort design will use a much larger percentage of the LSS cohort and should provide important results regarding the possible influence of smoking on the radiation risk estimates of UC. These results will be presented at the Radiation Research meeting in 2010 and an associated publication is also due in 2010. Preliminary results indicate that the radiation estimates are not greatly influenced by the presence of smoking or other lifestyle factors (Figure). The Figure shows excess cases of urothelial carcinomas/10,000/year. The radiation excess in both sexes is roughly the same whereas the smoking excess is much higher among men due to their high smoking prevalence.

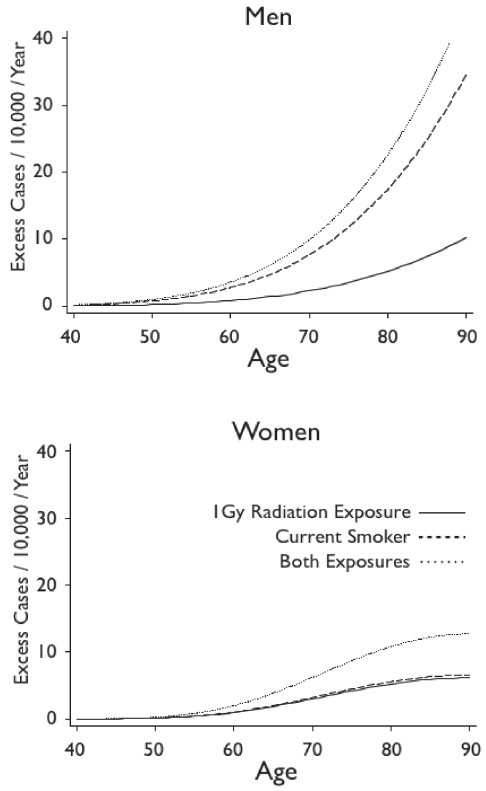


Figure. Excess cases of urothelial carcinomas/10,000/year

RP-A6-08 Analyses of lymphatic and hematopoietic cancer mortality

Richardson D, Sugiyama H (EH), Sakata R (EH), Grant EJ (EH), Shimizu Y (EH), Hsu WL (S), Soda M (EN), Suyama A (EN), Kodama K (CS), Kasagi F (EH), Ozasa K (EH)

The objective of this project is to investigate associations between ionizing radiation and mortality due to lymphatic and hematopoietic cancers using data obtained from Life Span Study (LSS) of atomic-bomb survivors. This study provides the first comprehensive report on mortality by type of leukemia among the Japanese atomic-bomb survivors in the LSS. Analyses include 310 deaths due to leukemia during the period 1950–2000 among 86,611 people in the LSS. Poisson regression methods were used to evaluate associations between estimated bone marrow dose and leukemia mortality. Attention was given to variation in the radiation dose-leukemia mortality association by time-since-exposure, age-at-exposure, city, and sex by types of leukemia. The excess relative rate per gray of acute myeloid leukemia was best described by a quadratic dose-response function that peaked approximately 10 years after exposure. Acute lymphatic leukemia and chronic myeloid leukemia mortality were best described by a linear dose-response function that did not vary with time since exposure. Adult T-cell leukemia was not associated with estimated bone marrow dose. Moreover, in the present study, the effect of the atomic bombings on leukemia mortality has persisted in this cohort for more than five decades. A paper was published in *Radiation Research* (Richardson et al., 2009; 172:368–82) and this research successfully terminated in 2009.

RP-A3-08 Potential confounding or interaction of anthropometric factors with radiation on colon cancer incidence among atomic bomb survivors in the Life Span Study (LSS) cohort

Semmens E, Li CI, Sugiyama H (EH), Moriwaki H (EH), Sakata R (EH), Soda M (EN), Kasagi F (EH), Yamada M (CH), Fujiwara S (CH), Akahoshi M (CN), Davis S, Kopecky KJ, Mabuchi K, Kodama K (CS)

The primary purpose of this project is to assess how anthropometric factors may confound or modify the relationship between radiation dose and risk of colon cancer incidence among atomic-bomb survivors in the Life Span Study (LSS) cohort. Anthropometric data obtained through questionnaires in 1965, 1969, 1978, and 1991 from members of the Life Span Study (LSS) cohort and from clinic records of members of the Adult Health Study (AHS) are being utilized. All persons enrolled in the LSS who completed at least one lifestyle questionnaire are included in the study. The outcome of interest is a diagnosis of colon cancer. Height, weight, body mass index (BMI), and radiation dose are the primary exposures of interest. Cox regression methods will be used to estimate the possible confounding or interactive effects of these anthropometric factors along with radiation. Preliminary analyses indicate 669 colon cancer cases that have BMI data from at least one questionnaire. These cases come from a pool of 1.2 million person-years (PYs) of data. The crude incidence rate for colon cancer among men was 74.5/100,000 PYs, while that of women was 45.4/100,000 PYs.

This RP was developed under the auspices of the

Radiation Research Partnership Program with the University of Washington and Kurume University. The RP was approved in October 2008. Ms. Semmens, a PhD student at the University of Washington, is carrying out the analyses and will write the manuscript. A manuscript is in preparation and will be submitted in 2010.

RP-A1-08 Bayesian MCMC applied to individual cancer incidence data in atomic bomb survivors

Little MP, Cullings HM (S), Furukawa K (S), Ozasa K (EH), Soda M (EN), Suyama A (EN), Sakata R (EH), Kasagi F (EH), Molitor J

The purpose of this protocol is to investigate the effects of errors in survivors' dose estimates on the estimation of radiation risk of cancer in the LSS, using Bayesian models with individual data under several models for assumed dose errors, and to compare the results to those expected from the theory associated with standard methods employed at RERF. It is well understood that measurement errors exist in DS02 dose estimates and that these affect risk estimates. Several researchers have investigated the problem with a view towards removing measurement-error bias in risk estimates. The method of dose adjustment currently employed at RERF is the so-called "regression calibration" method, which entails replacing the DS02 dose estimate with the expected value of the individual's true dose given their estimated dose. Although this approach leads to reasonable adjusted point estimates of risk parameters for linear dose-response models, it is an approximate method when used with non-linear dose-effect relationships and does not fully take account of the variability in risk estimates induced by the measurement errors.

Bayesian models can in principle incorporate a much wider variety of error models and can evaluate the resulting bias and imprecision of risk estimates using individual data rather than the grouped data typically used in the past at RERF. Because these models require evaluation of the likelihood by the computationally intensive method of Markov Chain Monte Carlo (MCMC) integration, computational requirements are an issue. Earlier, a student working under Dr. Little, Philip Li, found that computational requirements forced him to retreat from the full LSS data and work with a sub-sample using a nested case-control design, which he evaluated for a few key outcome measures such as leukemia and thyroid cancer incidence. During this fiscal year Dr. Furukawa began some small-scale simulations to compare ability to handle measurement error between an individual data model and a grouped data model with Poisson regression, which has historically been the main approach to risk assessment in RERF.

RP-A1-07 Analysis of lymphoma mortality

Richardson D, Sugiyama H (EH), Grant EJ (EH), Sakata R (EH), Geyer S (S), Shimizu Y (EH), Soda M (EN), Suyama A (EN), Kodama K (CS), Kasagi F (EH), Ozasa K (EH)

The objective of this project is to investigate associations between ionizing radiation and mortality due to lymphoma using data obtained from cohort mortality studies of workers employed at the Savannah River Site (SRS) and from the Life Span Study (LSS) of atomic-bomb survivors. The approach involves parallel analyses, in which study definitions and analytical methods are as comparable as possible. The relationships between ionizing radiation and lymphoma mortality among males in the LSS cohort who were 15–64 years of age at the time of the bombing, and male nuclear weapons workers who were hired at the SRS between 1950 and 1986 were investigated. Poisson regression methods were used to derive estimates of radiation dose-response association for death due to all malignant lymphomas and death due to non-Hodgkin's lymphoma. Temporal variation in the dose-response association was assessed by exposure time-window analysis. Positive associations between lymphoma mortality and radiation dose under a 5-year lag assumption were observed in both cohorts (ERR/Sv was 0.79 [90% CI: 0.10, 1.88] in the LSS and 6.99 [90% CI: 0.96, 18.39] in the SRS). In each cohort, evidence of a dose-response association was primarily observed more than 35 years after irradiation. These findings suggest a protracted induction and latency period for radiation-associated lymphoma mortality. A paper was published in the *American Journal of Epidemiology* (Richardson et al., 2009; 169:969–76) and this research successfully terminated in 2009.

Life Span Study Publications

RERF Reports (RR)

♦ Richardson DB, Sugiyama H, Nishi N, Sakata R, Shimizu Y, Grant EJ, Soda M, Hsu WL, Suyama A, Kodama K, Kasagi F: Ionizing radiation and leukemia mortality among Japanese atomic bomb survivors, 1950–2000. Radiation Research 2009 (September); 172(3):368–82.

[Abstract] This paper provides the first comprehensive report on mortality by type of leukemia among the Japanese atomic bomb survivors in the Life Span Study (LSS). Analyses include 310 deaths due to leukemia during the period 1950–2000 among 86,611 people in the LSS. Poisson regression methods were used to evaluate associations between estimated bone marrow dose and leukemia mortality. Attention was given to variation in the radiation dose-leukemia mortality association by time since exposure, age at exposure, city and sex. The excess relative rate per gray of acute myeloid leukemia was best described by a quadratic dose-response function that peaked approximately 10 years after exposure. Acute lymphatic leukemia and chronic myeloid leukemia mortality were best described by a linear dose-response function that did not vary with time since exposure. Adult T-cell leukemia was not associated with estimated bone marrow dose. Overall, 103 of the 310 observed leukemia deaths were estimated to be excess deaths due to radiation exposure. In the most recent decade of observation (1991–2000), the estimated attributable fraction of leukemia deaths among those survivors exposed to >0.005 Gy was 0.34, suggesting that the effect of the atomic bombings on leukemia mortality has persisted in this cohort for more than five decades.

♦ Richardson DB, Sugiyama H, Wing S, Sakata R, Grant EJ, Shimizu Y, Nishi N, Geyer SM, Soda M, Suyama A, Kasagi F, Kodama K: Positive associations between ionizing radiation and lymphoma mortality among men. American Journal of Epidemiology 2009 (April); 169(8):969–76.

Bloomberg School of Public Health (*This abstract was reprinted by permission of Oxford University Press.*) (RR 8-08)

[Abstract] The authors investigated the relation between ionizing radiation and lymphoma mortality in 2 cohorts: 1) 20,940 men in the Life Span Study, a study of Japanese atomic bomb survivors who were aged 15–64 years at the time of the bombings of Hiroshima and Nagasaki, and 2) 15,264 male nuclear weapons workers who were hired at the Savannah River Site in South Carolina between 1950 and 1986. Radiation dose-mortality trends were evaluated for all malignant lymphomas and for non-Hodgkin's lymphoma. Positive associations between lymphoma mortality and radiation dose under a 5-year lag assumption were observed in both cohorts (excess relative rates per sievert were 0.79 (90% confidence interval: 0.10, 1.88) and 6.99 (90% confidence interval: 0.96, 18.39), respectively). Exclusion of deaths due to Hodgkin's disease led to small changes in the estimates of association. In each cohort, evidence of a dose-response association was primarily observed more than 35 years after irradiation. These findings suggest a protracted induction and latency period for radiation-induced lymphoma mortality.

♦ Shimizu Y, Kodama K, Nishi N, Kasagi F, Suyama A, Soda M, Grant EJ, Sugiyama H, Sakata R, Moriwaki H, Hayashi M, Konda M, Shore RE: Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950–2003. British Medical Journal 2010; 340:b5349 (*This abstract was reprinted by permission of BMJ.*) (RR 4-09)

[Abstract] ***Objective.*** To investigate the degree to which ionizing radiation confers risk of mortality from heart disease and stroke. ***Design.*** Prospective cohort study with more than 50 years of follow-up. ***Setting.*** Atomic bomb survivors in Hiroshima and Nagasaki, Japan. ***Participants.*** 86,611 Life Span Study cohort members with individually estimated radiation doses from 0 to >3 Gy (86% received <0.2 Gy). ***Main outcome measures.*** Mortality from stroke or heart disease as the underlying cause of death and dose-response relations with atomic bomb radiation. ***Results.*** About 9,600 participants died of stroke and 8,400 died of heart disease between 1950 and 2003. For stroke, the estimated excess relative risk per gray was 9% (95% confidence interval 1% to 17%, P = 0.02) on the basis of a linear dose-response model, but an indication of possible upward curvature suggested relatively little risk at low doses. For heart disease, the estimated excess relative risk per gray was 14% (6% to 23%, P < 0.001); a linear model provided the best fit, suggesting excess risk even at lower doses. However, the dose-response effect over the restricted dose range of 0 to 0.5 Gy was not significant. Prospective data on smoking, alcohol intake, education, occupation, obesity, and diabetes had almost no impact on the radiation risk estimates for either stroke or heart disease, and misdiagnosis of cancers as circulatory diseases could not account for the associations seen. ***Conclusion.*** Doses above 0.5 Gy are associated with an elevated risk of both stroke and heart disease, but the degree of risk at lower doses is unclear. Stroke and heart disease together account for about one third as many radiation associated excess deaths as do cancers among atomic bomb survivors.

Commentary and Review Series (CR)

♦ Grant EJ, Shimizu Y, Kasagi F, Cullings HM, Shore RE: Radiation unlikely to be responsible for high cancer rates among distal Hiroshima A-bomb survivors. Environmental Health and Preventive Medicine 2009 (July); 14(4):247–9. © The Japanese Society for Hygiene 2009 (*The following first paragraph was reprinted by permission of Springer.*) (CR 1-09)

Watanabe et al. (hereafter referred to as WMHY) used A-bomb survivor data from the Radiation Effects Research Foundation (RERF) Life Span Study (LSS) Report 12 (available online at http://www.rerf.or.jp) and mortality rates for Hiroshima and Okayama Prefectures to create standardized mortality ratios (SMR) for LSS cohort members residing in Hiroshima at the time of the bombing. WMHY divided cohort members into three radiation dose categories: very low dose (<0.005 Gy, VLD), low dose (0.005–0.1 Gy, LD), and high dose (>0.1 Gy, HD). At issue are the men in the VLD category, who experienced higher than expected deaths due to cancers compared with prefectural rates (note that, for brevity, we discuss only the solid cancer results). WMHY concluded that the increased cancer deaths among the VLD were due to underestimated neutron doses and/or unaccounted-for residual radiation exposures. We find their conclusions to be implausible and believe that the data show it is far more likely that the observed risks among the VLD are due to nonradiation factors. The reasons are briefly summarized below.

Other Journal Publications

♦ Darby SC, Cutter DJ, Boerma M, Constine LS, Fajardo LF, Kodama K, Mabuchi K, Marks LB, Mettler FA, Pierce LJ, Trott KR, Yeh ETH, Shore RE: Radiation-related heart disease: Current knowledge and future prospects. International Journal of Radiation Oncology, Biology, Physics 2010 (March); 76(3):656–65. (related to *Adult Health Study*)

♦ Fujiwara S, Yamada M, Takahashi I: Current status of large-scale epidemiological study—Adult Health Study in Hiroshima and Nagasaki. Japanese Journal of Stroke 2009 (November); 31(6):439–42. (Japanese)

Manuscript in Press

⌘ Grant EJ, Ozasa K: Etiology of cancer/ionizing radiation. Cancer Report of Asian-Pacific Region 2010. (Supplement for the meeting of the Fifth International Asian Pacific Organization for Cancer Prevention [APOCP], Istanbul, Turkey, 3–7 April 2010)

⌘ Hsu WL, Tatsukawa Y, Neriishi K, Yamada M, Cologne JB, Fujiwara S: Longitudinal trends of total white blood cell and differential white blood cell counts of atomic bomb survivors. Journal of Radiation Research. (related to *Special Clinical Studies*)

Life Span Study Oral Presentations

❖ Nishi N, Sugiyama H, Sakata R, Grant EJ, Soda M, Kasagi F, Suyama A, Ozasa K, Nagano J. A new Mail Survey on the Life Span Study cohort. Late Health Effects of Ionizing Radiation, 4–6 May 2009, Washington DC, USA

❖ Richardson DB, Sugiyama H, Nishi N, Sakata R, Shimizu

Y, Grant EJ, Soda M, Hsu WL, Suyama A, Kodama K, Kasagi F. Ionizing radiation and leukemia mortality among Japanese atomic-bomb survivors, 1950–2000. Late Health Effects of Ionizing Radiation, 4–6 May 2009, Washington DC, USA

❖ Sakata R, Shimizu Y, Nishi N, Sugiyama H, Hayashi M, Soda M, Ozasa K. Effects of other factors in radiation risk assessment of gynecologic cancer incidence. Late Health Effects of Ionizing Radiation, 4–6 May 2009, Washington DC, USA

❖ Semmens E, McDougall J, Grant EJ, Nishi N, Kodama K, Suyama A, Ozasa K, Davis S, Kopecky KJ, Li CI. Acute radiation exposure and risk of second primary cancers in atomic-bomb survivors. The 21st Annual Conference of the International Society for Environmental Epidemiology, 25–28 August 2009, Dublin, Ireland

❖ Ozasa K, Shimizu Y, Suyama A, Kasagi F, Nishi N, Soda M, Grant EJ, Sakata R, Sugiyama H, Kodama K. Overview of mortality in the Life Span Study during 1950–2003. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Samartzis D, Nishi N, Cologne JB, Hayashi M, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F. The association of low to moderately high-levels of ionizing radiation exposure and the development of soft tissue sarcomas. The 29th Annual Congress of the Hong Kong Orthopaedic Association, 28–29 November 2009, Hong Kong, China (related to *Tumor and Tissue Registry*)

❖ Kodama K, Shimizu Y, Shore RE. Radiation and cardiovascular disease—Findings from the atomic-bomb survivors. Kids Workshop 2009 in National Institute of Radiological Sciences, 14–17 December 2009, Chiba (related to *Adult Health Study*)

❖ Shore RE. Risk of solid cancers in children exposed to the atomic-bombs. Kids Workshop 2009 in National Institute of Radiological Sciences, 14–18 December 2009, Chiba (related to *Tumor and Tissue Registry*)

❖ Ozasa K, Shimizu Y, Nishi N, Soda M, Grant EJ, Sakata R, Sugiyama H, Nonaka Y, Kasagi F, Suyama A. Birth cohort effect on cancer mortality in Japan, 1950–2006. The Joint Scientific Meeting of IEA Western Pacific Region and the 20th Japan Epidemiological Association, 9–10 January 2010, Koshigaya

Research Protocols 7-09, 3-07, 2-75 (Platform Protocol), A3-09

Adult Health Study (AHS)

RP 7-09 Study of arteriosclerosis in the Adult Health Study population (Part I. Physiological indexes of arteriosclerosis)

Takahashi I (CH), Hida A (CN), Akahoshi M (CN), Kohata M, Yamada M (CH), Hsu WL (S), Misumi M (S), Takahashi T, Kihara Y, Matsumoto M, Fujiwara S (CH)

The goal is to study one of the potential mechanisms by which radiation may promote cardiovascular disease. Past studies have reported a significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors. Atherosclerosis conceptually has two aspects: atherosis (the fatty degeneration) and sclerosis (arterial stiffness). We will evaluate acceleration of arterial stiffness by radiation among AHS subjects including the expanded group of younger survivors. While acceleration of arterial stiffness might be caused by radiation-induced structural changes in arterial walls, it has not been fully investigated. In this study, we will evaluate the associations of radiation and arterial stiffness taking into account correlations among stiffness indices and atheromatous disease indices/risk factors both in Hiroshima and Nagasaki.

This is a cross-sectional study among all AHS subjects in Hiroshima and Nagasaki. The associations of radiation and the arterial stiffness indices (brachial-ankle pulse wave velocity [baPWV], augmentation index [AI]) will be analyzed with consideration with atheromatous disease indices (ankle-brachial blood pressure index [ABI], intima-media wall thickness [IMT], aortic calcification, and left ventricular hypertrophy) and atherosclerosis risk factors (Framingham risk scores).

This protocol was approved at the end of 2009, and we will start to measure these indices among AHS subjects in April 2010.

RP 3-07 Clinical health study for expanded group of younger A-bomb survivors

Akahoshi M (CN), Yamada M (CH), Hida A (CN), Ohishi W (CH), Nishi N (EH), Kasagi F (EH), Suyama A (EN), Furukawa K (S), Cullings HM (S), Hayashi T (R), Nakachi K, Kodama Y (G), Katayama H (IT), Kodama K (CS), Nakamura N (CS), Fujiwara S (CH)

LSS data show that those exposed to A-bomb radiation at a young age have a greater cancer risk than those exposed when older. AHS data show similar results for benign thyroid tumors, hyperparathyroidism, hepatitis B virus (HBV) infection, and myocardial infarction. By expanding the cohort of younger survivors, we will enhance the statistical power and precision for estimating risks among those who were young at the time of the bombing.

The existing AHS cohort consisted of all identified heavily exposed individuals and a small fraction of those lightly or moderately exposed. Adding more of the latter to the AHS cohort will provide a much better assessment of low and moderate radiation dose effects in younger subjects and will increase the number of biological samples from younger survivors for molecular biological studies

in the future.

Since subjects who received the lowest doses (<5 mGy) are already the largest group of AHS subjects, little statistical power would be gained by the addition of many more such subjects. We therefore plan to solicit 30% of the potentially eligible who were exposed to <5 mGy, 80% of those exposed to 5–20 mGy, and all exposed to 20–1,000 mGy which will add up to 2,300 subjects of whom the majority received 20–1,000 mGy. We will focus on non-cancer disease (liver disease, thyroid disease, ophthalmologic disease, and cardiovascular disease) in addition to cancer.

We have examined 1,268 subjects as of October 31, 2009, and will examine approximately 1,800 subjects as of June 31, 2010 in Hiroshima and Nagasaki. Because the initial participation rate was lower than expected, the total number of subjects will be somewhat decreased from our initial plans for 2,300 subjects, but fortunately it has very little effect on the statistical power.

RP 2-75 Research plan for RERF Adult Health Study, Hiroshima and Nagasaki

Fujiwara S (CH), Neriishi K (CH), Yamada M (CH), Ohishi W (CH), Tatsukawa Y (CH), Takahashi I (CH), Akahoshi M (CN), Hida A (CN), Sera N (CN), Imaizumi M (CN), Soda M (EN)

The AHS program was initiated to provide the first scientific information on the long-term clinical health consequences of radiation exposure to the general population. It continues to provide unique data addressing that objective. To evaluate in a systematic fashion the age and radiation exposure-dependent changes in the clinical status of long-term survivors (AHS cohort) of the atomic bombings, and to provide extensive biological specimen and information concerning lifestyle or other potential risk factors for many fields of study, which include cytology, genetics, immunology, radiobiology, and medical dosimetry.

The AHS program of biennial comprehensive medical examinations began in 1958 with a targeted population of about 20,000 survivors and controls in the contact areas of Hiroshima and Nagasaki. In 1978, the sample was enriched with about 2,400 additional higher-dose subjects and all available (~1,000) persons who were exposed *in utero,* while about 5,000 not-in-city study subjects were dropped as being largely duplicative with other unexposed study subjects. To provide a better assessment of radiation effects among those young at exposure, we are currently adding more young exposed subjects (<10 years old at the bombings) to the study.

The study attempts to examine differences in the prevalence or incidence of diseases or pre-clinical disorders among those with a wide range of radiation exposures. During the 25th cycle of the AHS (July 2006–June 2008), a total of 3,609 individuals were examined, representing approximately 70% of the AHS cohort still living in the contact areas of interest.

A new cycle of health examinations of the original AHS group and the newly added group is nearing completion. The biological specimens collected are used for clinical determinations and stored for future studies. Research protocols using stored specimens are being conducted to evaluate possible interactions between radiation and infectious agents or hormones and cancer risk, and phenotypic and genetic factors associated with inflammation and myocardial infarction. Tissue collection and storage of operated cataract tissue was begun in 2009. Other recently initiated radiation-related studies include ones on; liver stiffness, chronic kidney dysfunction/disease and risk of cardiovascular disease, and pre-clinical measurements of atherosclerosis.

Reports of new findings include; radiation and lifetime risk of stroke, radiation and dementia, metabolic cardiovascular risk factors and subclinical hypothyroidism, genotypes associated with diffuse type noncardia gastric cancer, and biological age and mortality.

RP-A3-09 The association between chronic kidney disease and cardiovascular disease among atomic bomb survivors

Tsuneto A, Takahashi I (CH), Hida A (CN), Sera N (CN), Imaizumi M (CN), Yamada M (CH), Neriishi K (CH), Ohishi W (CH), Tatsukawa Y (CH), Nakashima E (S), Hsu WL (S), Misumi M (S), Fujiwara S (CH), Akahoshi M (CN)

The association between atomic-bomb radiation exposure and cardiovascular disease (CVD) has recently been drawing attention. A-bomb radiation exposure has been reported to be associated with a number of CVD risk factors. Chronic kidney disease (CKD) has recently been recognized as a risk factor for CVD. CKD and CVD share many common risk factors such as obesity, insulin resistance, impaired glucose tolerance, hypertension, dyslipidemia, and nephritis. No study has been conducted at RERF thus far as to whether CKD is related to radiation, and whether CKD might serve as a mediating variable in the association of radiation with CVD.

In this analysis, we will identify prevalent cases of CKD diagnosed during the four-year baseline period (1988–91) and incident cases of CKD diagnosed during the 15-year follow-up period of 1992–2006 in the AHS cohort. We will also identify both prevalent and incident cases of CVD during the above-mentioned periods, respectively. CVD includes coronary heart disease (CHD) and stroke. Based on these data, we will determine whether the effects of A-bomb radiation exposure can be observed for several endpoints, with adjustment for other risk factors:

(1) Associations of prevalent cases of CKD with radiation dose and CKD risk factors.
(2) Associations of incident cases of CKD with radiation dose and CKD risk factors.
(3) Associations of prevalent cases of CHD and stroke with radiation dose, CKD risk factors and prevalent cases of CKD.

Associations of incident cases of CHD and stroke with radiation dose, CKD risk factors, and prevalent cases of CKD. We have started this RP February 2010.

Adult Health Study Publications

Journal Publications

♦ Akahoshi M: Ischemic heart disease among atomic bomb survivors: Possible mechanism(s) linking ischemic heart disease and radiation exposure. Nakashima M et al. eds. Radiation Health Risk Sciences. Proceedings of the First International Symposium of the Nagasaki University Global

COE Program "Global Strategic Center for Radiation Health Risk Control." New York: Springer; 2009, pp 63–8.

♦ Darby SC, Cutter DJ, Boerma M, Constine LS, Fajardo LF, Kodama K, Mabuchi K, Marks LB, Mettler FA, Pierce LJ, Trott KR, Yeh ETH, Shore RE: Radiation-related heart disease: Current knowledge and future prospects. International Journal of Radiation Oncology, Biology, Physics 2010 (March); 76(3):656–65. (related to *Life Span Study*)

Manuscripts in Press

⌘ Blakely EA, Kleiman NJ, Neriishi K, Chodick G, Chylack LT, Cucinota FA, Minamoto A, Nakashima E, Kumagami T, Kitaoka K, Kanamoto T, Kiuchi Y, Chang P, Fujii N, Shore RE: Meeting report: Radiation cataractogenesis: Epidemiology and biology. Radiation Research. (related to *Special Clinical Studies*)

⌘ Fujiwara S: Path traveled by the Radiation Effects Research Foundation's research. Hiroshima Igaku [Journal of the Hiroshima Medical Association] (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

Adult Health Study Oral Presentations

❖ Fujiwara S. "History of the Late A-bomb Effects Research Society" Path traveled by the Radiation Effects Research Foundation's research. The 50th Late A-bomb Effects Research Meeting, 7 June 2009, Hiroshima

❖ Nakashima E, Neriishi K, Akahoshi M, Grant EJ, Preston DL, Masunari N, Funamoto S. Cataract surgery incidence in atomic-bomb survivors: 1986–2005. The 50th Late A-bomb Effects Research Meeting, 7 June 2009, Hiroshima

❖ Kusunoki Y, Kubo Y, Yamaoka M, Hamasaki K, Hayashi T, Imai K, Nakachi K. Evaluation of radiation-induced genetic instability in the hematopoietic system by reticulocyte micronucleus frequency analysis among A-bomb survivors. The 19th Annual Meeting of the Japan Cytometry Society, 20–21 June 2009, Matsue

❖ Nakashima E, Neriishi K, Akahoshi M, Grant EJ, Preston DL, Masunari N, Funamoto S. Cataract surgery incidence in atomic-bomb survivors: 1986–2005. The 55th Annual Meeting of the Radiation Research Society, 4–7 October 2009, Savannah, Georgia, USA

❖ Ohishi W, Tatsukawa Y, Fujiwara S, Masunari N, Yamada M, Tsuge M, Chayama K. Relationship between metabolic syndrome and liver dysfunction and/or fatty liver. The 13th Annual Meeting of the Japan Society of Hepatology, 14–17 October 2009, Kyoto (related to *Special Clinical Studies*)

❖ Takahashi I, Geyer SM, Fujiwara S, Nishi N, Kodama K, Ohshita T, Takahashi T, Matsumoto M. Lifetime risk of stroke in atomic-bomb survivors—Adult Health Study in Hiroshima and Nagasaki, Japan. American Heart Association Scientific Sessions 2009, 14–18 November 2009, Orlando, Florida, USA

❖ Kodama K, Shimizu Y, Shore RE. Radiation and cardiovascular disease—Findings from the atomic-bomb survivors. Kids Workshop 2009 in National Institute of Radiological Sciences, 14–17 December 2009, Chiba (related to *Life Span Study*)

Research Protocol 1-02
F_1 Clinical Study (FOCS)—Platform Protocol

RP 1-02 Health effects study of the children of A-bomb survivors: Clinical health study

Fujiwara S (CH), Tatsukawa Y (CH), Suyama A (EN), Cologne JB (S), Akahoshi M (CN), Yamada M (CH), Suzuki G, Koyama K, Takahashi N (G), Kasagi F (EH), Grant EJ (EH), Lagarde F, Hsu WL (S), Furukawa K (S), Ohishi W (CH), Neriishi K (CH), Takahashi I (CH), Ashizawa K, Hida A (CN), Imaizumi M (CN), Nagano J, Cullings HM (S), Katayama H (IT), Ross NP (S), Kodama K (CS), Shore RE (D)

The F_1 Clinical Study (FOCS) is intended to assess the possible genetic effects and associated long-term health consequences among children of Japanese parents who were acutely exposed to ionizing radiation from the atomic-bomb detonations over Hiroshima and Nagasaki. The hypothesis behind the FOCS is that ionizing radiation may induce genetic mutations in germ cells that cause hereditary diseases, in particular, adult-onset multifactorial diseases including diabetes, essential hypertension, coronary heart disease, stroke, etc. The study has three major objectives: (1) to investigate the effects of parental exposure to A-bomb radiation on the development of multifactorial diseases among the children of A-bomb survivors, (2) to preserve blood samples for future molecular biological studies, and (3) to contribute to the health and welfare of the F_1 population via health examinations and health guidance. A total of 11,951 participated in the health examination during the study period from 2002 to 2006. The participation rate in the mail survey cohort was 48.4% (43% for males and 55% for females; 49% for Hiroshima and 48% for Nagasaki). The clinical assessment of nearly 12,000 offspring of A-bomb survivors provided no evidence for an increased prevalence of adult-onset multifactorial diseases in relation to parental radiation exposure. The paper entitled "Prevalence of adult onset multifactorial disease among offspring of atomic-bomb survivors" was published in *Radiation Research* (Fujiwara et al., 2008; 170:451–57) (Table).

Table. Adjusted prevalence odds ratio for multifactorial disease among offspring in relation to parental radiation dose

	Odds ratio at 1 Gy (95% confidence interval)*		
	All participants	Male offspring	Female offspring
Father's dose	0.91 (0.81–1.01)	0.76 (0.65–0.89)	1.04 (0.90–1.21)
Mother's dose	0.98 (0.86–1.10)	0.97 (0.81–1.17)	0.98 (0.83–1.16)
Sum of both doses	0.94 (0.86–1.02)	0.85 (0.75–0.96)	1.02 (0.91–1.13)

* Odds ratios were adjusted for categories of age, sex, city, body mass index, parental history of multifactorial disease, female menopause, smoking, alcohol intake, and occupation.

Analyses of genetic effects on individual multifactorial diseases such as hypertension, hypercholesterolemia, and diabetes are ongoing with the collaboration of the Departments of Statistics, Epidemiology, and Genetics.

The detailed research plan for a F_1 clinical follow-up study has been discussed in the F_1 Clinical Follow-up Study Research Group and research plans for a long-term health study of F_1 individuals are being prepared.

Research Protocols 5-09, 4-09, 3-09, 4-04 and 5-04, 1-03, 4-02, 2-97, 1-93, 2-90, 7-87, 3-87

Immunology Studies

RP 5-09 Effects of radiation exposure and aging on hematopoietic stem cells (HSCs) and dendritic cells (DCs) —Analyses of numerical and functional changes

Kusunoki Y (R), Yoshida K (R), Hayashi T (R), Geyer S, Misumi M (S), Ohishi W (CH), Fujiwara S (CH), Ozasa K (EH), Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng NP, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

This study aims to delineate the long-term consequences of prior A-bomb irradiation and advancing age on homeostatic control of HSCs and DCs. Based on accumulating evidence for accelerated immunosenescence in A-bomb survivors, we initiated an international collaboration study on mechanisms of radiation-related immunosenescence, with support by funding from the U.S. National Institute of Allergy and Infectious Diseases (NIAID). In that study, we hypothesize that radiation exposure induces premature aging of HSCs, resulting in reduced numbers and impaired self-renewal ability, that in turn accelerate loss of lymphoid potential. We also hypothesize that A-bomb irradiation affects innate and adaptive immunity, possibly by altering DC populations toward a T-cell suppressor phenotype. Numerical and functional changes in relation to radiation dose will be analyzed within the circulating HSC and DC pools among several hundred individuals who are currently participating in the Hiroshima AHS. In order to confirm the results of the A-bomb survivor studies, we will develop a series of *in vitro* and *in vivo* assay systems to determine the functional and differentiation status of HSC and DC populations following ionizing irradiation.

We have started the development and validation of assays for the numbers and functions of human peripheral blood HSC and DC populations at RERF. Using a number of mouse models, we will investigate the processes of hematopoietic and immune reconstitution following radiation-induced damage, in order to understand how irradiation modulates hematopoietic functions, myeloid and lymphoid cell differentiation, inflammatory responses, and genomic stability. Effects of radiation and aging on bone marrow stromal and thymic epithelial cells will also be analyzed in the mouse models. We will also analyze the characteristics and functions of hematolymphoid cells reconstituted in irradiated hosts; this approach will include investigations in SCID-hu mice containing human blood cells.

RP 4-09 Effects of ionizing radiation exposure and aging on vaccination responses

Hayashi T (R), Kusunoki Y (R), Imai K (R), Yoshida K (R), Ito R (R), Ohishi W (CH), Fujiwara S (CH), Ozasa K (EH), Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng NP, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

The RERF's epidemiology and clinical studies have long

indicated increased risks of age-related and immune system/inflammation-related diseases among A-bomb survivors. Further, the noted radiation effects on the immune system are similar to those associated with aging. It is important to examine whether the radiation-impaired immune system modifies the response to vaccination. The purpose of this study is to evaluate the effects of prior A-bomb radiation exposure on the immunological capacity of aging individuals to respond to influenza vaccination. We will recruit 50 AHS subjects for a pilot study and 300 AHS subjects for the full-scale study by stratified random sampling on dose, age, and gender. Collection and storage of blood serum, plasma, and lymphocyte samples will be conducted immediately before and 3 weeks after the vaccination. The primary endpoint will be the change in anti-influenza virus antibody titer levels from before to 3 weeks after vaccination. Secondary endpoints to be analyzed include levels of cytokines and inflammation-related proteins, lymphocyte subsets, and intracellular activation markers (mRNA and protein). Those parameters will be analyzed in relation to age and dose of prior radiation exposure.

This RP was approved in August 2009, and an assay validation with 20 in-house volunteers has been started.

RP 3-09 Development of an integrated scoring system for human immune competence as it relates to age and ionizing radiation

Hayashi T (R), Kusunoki Y (R), Imai K (R), Yoshida K (R), Ito R (R), Ohishi W (CH), Fujiwara S (CH), Ozasa K (EH), Furukawa K (S), Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng NP, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

The immunology study unique to RERF constitutes repeated observations of various immunological parameters in A-bomb survivors with long-term follow-up, demonstrating significant radiation-related alterations in the immune system among survivors, found even today. The objective of this study is to develop an integrated scoring system for evaluating immunological and inflammatory status of individuals as a function of age and radiation dose. This study consists of a cross-sectional analysis and a longitudinal analysis. The proposed cross-sectional analysis will include about 3,600 Hiroshima AHS subjects. Measurements will be made in plasma and blood of the immunological and inflammation-related markers. The proposed longitudinal analysis will include a random subset of 300 AHS subjects. Biomarkers will be measured on two sets of plasma samples collected from the 300 AHS subjects ten years apart, using a multiple assay system for the simultaneous quantitative determination of plasma cytokines. Telomere-length assays will also be conducted on DNA from the same 600 samples. The results will be utilized to construct an integrated scoring system that effectively will reflect overall immune-related health, and how that immune status differs across varying age and radiation-exposed groups.

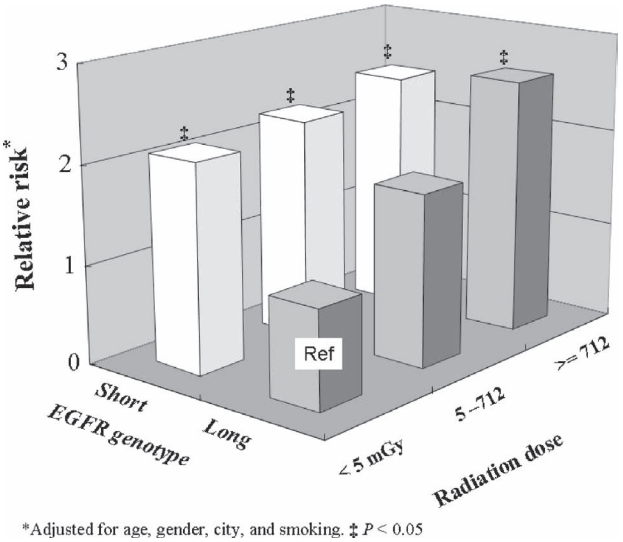
This RP was approved in August 2009, and the measurements of biomarkers have started. Plasma levels of IL-6, TNF-α, IL-10, and CRP in 2,000 Hiroshima AHS subjects have already been measured. In this fiscal year, we started examining plasma levels of those biomarkers, frequencies of lymphocyte subsets, and oxidative stress markers such as intracellular and plasma ROS levels using blood samples from the AHS subjects.

RP 4-04 Relationship between cancer development and genetic polymorphisms among A-bomb survivors, focusing on immune-related genes
RP 5-04 Identification of cancer-related gene polymorphisms and immunological markers (Addendum to RP 4-04)

Hayashi T (R), Morishita Y (R), Nagamura H (R), Maki M (R), Kusunoki Y (R), Yoshida K (R), Imai K (R), Cologne JB (S), Tahara E, Fujiwara S (CH), Akahoshi M (CN), Nakachi K

Epidemiological studies have clearly demonstrated long-lasting impacts of A-bomb radiation on human health, including dose-dependent increases in the incidence/mortality of inflammation-related cancers. Although enhanced inflammation has been consistently observed among A-bomb survivors, roles of inflammatory responses in radiation carcinogenesis are not understood. The purpose of this study is to assess whether genetic backgrounds of individuals affect their susceptibility to cancer, particularly as this may be a modifier of the radiation response. The study will focus on polymorphisms of genes encoding molecules that are possibly involved in immunological defenses against cancer development or in inflammatory responses that may modify cancer risk. A preliminary study showed that the risk of the intestinal-type gastric cancer was modulated primarily by *IL-10* haplotypes, while the risk of the diffuse-type gastric cancer was affected by both *IL-10* haplotypes and radiation exposure at a high radiation dose. We also found a synergistically increased risk of colon cancer for those with a certain *IL-18* genotype and high radiation exposures.

In this fiscal year, we carried out a case-cohort study to evaluate a possible association of an *EGFR* CA repeat polymorphism with lung cancer risk in radiation-exposed or non-exposed A-bomb survivors. First, by dividing study



*Adjusted for age, gender, city, and smoking. ‡ $P < 0.05$

Figure. Lung cancer risk for radiation dose combined with *EGFR* CA repeat genotypes

subjects into *Short* (the number of CA repeats ≤ 37) and *Long* (≥38 repeats) genotypes, we found that the *Short* genotype was significantly associated with an increased risk of lung cancer, specifically adenocarcinoma, among non-exposed subjects. Next we found that prior radiation exposure significantly enhanced lung cancer risk of survivors with the *Long* genotype, while the risk for the *Short* genotype did not show any significant increase with radiation dose, resulting in indistinguishable risks between these genotypes at a high radiation dose (Figure). Our findings imply that the EGFR pathway appears to play a complex role in affecting individual susceptibility to lung adenocarcinoma in relation to radiation exposure.

RP 1-03 A study of gene polymorphisms and their possible role in the development of diabetes in the Adult Health Study population

Hayashi T (R), Morishita Y (R), Nagamura H (R), Yoshida K (R), Kusunoki Y (R), Nakashima E (S), Tatsukawa Y (CH), Fujiwara S (CH), Akahoshi M (CN), Imai K (R), Nakachi K

Although early studies of A-bomb survivors did not show associations between radiation exposure and a risk of diabetes, data on AHS subjects in 1992–1994 indicated a significant positive radiation-diabetes association in Hiroshima but not in Nagasaki (radiation dose-city interaction; $p < 0.001$), after adjusting for sex, age, and body mass index. This somewhat puzzling finding may reflect genetic differences between the Hiroshima and Nagasaki populations. Our preliminary results suggest that radiation may persistently impair immune responses, and that the radiation-diabetes association is especially relevant to a sub-group of A-bomb survivors who have a specific *HLA* class II haplotype. That observation suggests that the effects of radiation on the development of diabetes may vary according to genetic backgrounds. The purpose of the study is to assess the effect of radiation and various genetic factors on the risk of diabetes mellitus (DM) in terms of a case-control study within the AHS cohort, and determine whether differences in frequencies of any particular genotypes between Hiroshima and Nagasaki survivors may account for why a significant association between risk of DM and radiation dose is observed in Hiroshima survivors but not in Nagasaki survivors.

This fiscal year, we identified *DQA1* and *DRB1* genotypes for >99% of 913 DM patients and 2,458 controls. We are analyzing the relationships between *HLA*-related genetic factors, risk of diabetes, and radiation dose in the identified participants.

RP 4-02 Perturbation of T-cell homeostasis in atomic-bomb survivors

Kusunoki Y (R), Yoshida K (R), Hayashi T (R), Fujiwara S (CH), Kasagi F (EH), Hamasaki K (G), Kodama Y (G), Nakachi K

We wish to examine the proposition that radiation exposure can seriously perturb one or more of the processes involved in T-cell homeostasis in humans. The type and extent of any substantial impairment (or in some situations, imbalance) of immunological defense systems may well be an important element in excessive, and perhaps ongoing, cancer and non-cancer disease processes. Our basic strategy involves studying the blood leukocytes of about 1,000 A-bomb survivors who are current or recent participants in the AHS population. Those leukocyte evaluations will determine (1) numbers of T lymphocytes that contain T-cell receptor-rearrangement excision circles (TRECs; an indicator of production of new, naïve T cells), (2) average lengths of telomere repeats in T lymphocytes (an indicator of T-cell senescence), and (3) composition of lymphocyte subsets and other immunity-determining cell populations.

To date, we have found dose-dependent reductions in sizes of naïve T-cell populations, as well as increased percentages of memory T-cell populations, with radiation dose among study subjects. Preliminary analyses showed that the number of lymphocytes containing TRECs in the CD4 T-cell fraction tended to decrease with age as well as with radiation dose. We also observed a similar decreasing trend (i.e., age- or dose-associated decrease) in telomere lengths of CD8 T-cell populations among the survivors. In addition, we found a dose-dependent increase in the percentages of functionally weak memory subsets and regulatory T cells in the CD4 T-cell population of the survivors. Those results are consistent with the hypothesis that A-bomb radiation exposure may have perturbed T-cell homeostasis and accelerated immunosenescence.

To test our hypothesis that perturbation of T-cell homeostasis causes enhanced inflammatory responses, we further examined associations between inflammatory biomarkers and percentages of T-cell subpopulations. There was an inverse association between plasma levels of TNF-α and percentages of naïve CD4 T cells, but not those of regulatory T cells, among study subjects (Figure). The results suggest that impaired maintenance of naïve CD4 T-cell pool may result in activation of inflammatory responses in A-bomb survivors.

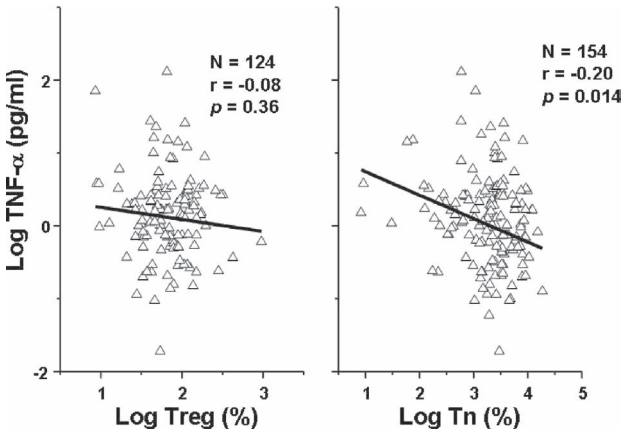


Figure. An inverse association between plasma TNF-α levels and percentages of naïve T (Tn) but not regulatory T (Treg) cells among A-bomb survivors.

RP 2-97 Lyophilization of blood samples for DNA extraction to be obtained from Adult Health Study subjects in Hiroshima and Nagasaki (Addendum to RP 2-90)

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Fujiwara S (CH), Nakachi K

For studying late effects of radiation exposure in A-bomb survivors, analyses of gene alterations due to radiation are essential in investigations of genetic instability, genetic susceptibility, and molecular oncology. With new technologies, the investigations can be conducted using extremely small amounts of DNA. This RP was designed to supplement RP 2-90 by providing a means of storing DNA for multiple, small-scale molecular analyses that otherwise would be wasteful of stored DNA from stock sources (i.e., biospecimens from RP 2-90). Blood samples, destined for DNA extraction and subsequent molecular analyses, are lyophilized and stored for use in current and future studies.

Blood remaining after clinical examination is used in this study. As the Immunogenome Studies including SNP analyses have started with a requirement of large amounts of DNA, as many nucleated cells as possible are harvested and directly stored at –80°C. Since the erythrocyte sedimentation test conducted in the Department of Clinical Studies was terminated in April 2008, additional blood cells are available for storage. We have so far cryopreserved 16,841 blood samples from 3,437 AHS participants in Hiroshima and 10,633 from 2,534 AHS participants in Nagasaki. It was determined by PCR amplification that DNA extracted from laboratory control blood stored at –80°C for 12 years on paper was not significantly affected by storage. We have also collected 1,310 blood cell samples (890 in Hiroshima and 420 in Nagasaki) from the expanded group of young A-bomb survivors since December 2007.

RP 1-93 Repertoire of T-cell antigen receptors and activity of hematopoietic progenitor cells in peripheral blood of atomic-bomb survivors (Addendum to RPs 7-89 [terminated], 4-87 [terminated], and 3-87)

Hayashi T (R), Kusunoki Y (R), Ohishi W (CH), Fujiwara S (CH), Kasagi F (EH), Yoshida K (R), Imai K (R), Nakachi K

A follow-up of possible radiation-induced alterations in blood cell populations of the survivors has been carried out to further elucidate the late effects of A-bomb radiation on the lymphohematopoietic system and their relationship to selected diseases being observed in the A-bomb survivors. The purpose of this study is to examine radiation effects on the hematolymphoid system related to inflammation and genomic instability that might lead to the development of aging-associated diseases including cancer. We hypothesize that exposure to A-bomb radiation has induced significant abnormalities in T-cell function and changed the proportion of some T-cell subsets in A-bomb survivors, resulting in chronic inflammation that has been enhanced more than in non-exposed individuals.

To investigate the relationships between oxidative stress and inflammatory status, we have developed an intracellular reactive oxygen species (ROS) assay system by flow cytometry. Intracellular ROS was measured in granulocytes, monocytes, and lymphocyte subsets (naïve CD4, memory CD4, naïve CD8, and memory CD8 T cells) obtained from 1,834 AHS subjects (Hiroshima 680 and Nagasaki 490 AHS subjects plus Hiroshima 428 and Nagasaki 236 AHS subjects from the newly expanded group of younger A-bomb survivors) in this fiscal year. Those measurements are ongoing. We have also evaluated a multiple assay system with the Bio-Plex for the simultaneous quantitative determination of plasma IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, and GM-CSF levels, using 500 control samples.

RP 2-90 Cryopreservation of blood cells from Hiroshima and Nagasaki Adult Health Study participants (See also RP 2-97)

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Fujiwara S (CH), Nakachi K

Since A-bomb survivors are now reaching advanced ages, we propose to cryopreserve live blood cells from all AHS participants to ensure a readily available source of materials for future studies. Lymphocytes of approximately 7,000 survivors have been preserved, and medical histories of these subjects have been recorded at the Department of Clinical Studies. About 1,500 of those subjects developed cancers before or after preservation of their lymphocytes. Biological materials from about 2,000 subjects have been used to assess the effects of A-bomb radiation on various endpoints, including a range of immune functions in addition to somatic mutations. Molecular epidemiological analysis of genetic susceptibility to cancer and other diseases is one of the most important research areas that we wish to pursue in the future. Blood samples collected from the same individuals at different times (e.g., approximately 10-year intervals) will permit the conduct of a unique longitudinal study to assess age and joint age-by-radiation effects on various blood biomarkers. To facilitate such studies, we will collect as many blood samples as possible from current AHS participants and cryopreserve mononuclear cells isolated from these samples.

We have cryopreserved blood cells from 7,106 AHS participants (4,528 in Hiroshima and 2,578 in Nagasaki) and 1,256 AHS participants from the newly expanded group of younger A-bomb survivors (836 in Hiroshima and 420 in Nagasaki) until November 2009. We confirmed that the viability of cryopreserved cells was more than 80% and that thawed lymphocytes expressed surface antigens and immunological functions as expected for live lymphocytes.

RP 7-87 X-ray radiosensitivity of lymphocytes *in vitro* from A-bomb survivors. Part 3: Transformation of B cells by Epstein-Barr virus and their cryopreservation (Addendum to RP 3-86 [terminated])

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Fujiwara S (CH), Nakachi K

For this study, it was initially proposed to cryopreserve EBV-transformed B-cell lines from high-dose and control survivors for new cell biology studies, e.g., those dealing with radiosensitivity. However, it soon became obvious that the resulting B-cell lines would be of considerable use in a variety of other research fields, including and perhaps especially those involving alterations to immune functions as well as those in which the role of genetic background in disease development is being investigated. Cell lines from 807 Hiroshima AHS participants have been cryopreserved for future studies since this project began in 1987. Following a recommendation by the Multinational Peer Review Panel, we began to accelerate EBV transformation of lymphocytes

from AHS participants in June 1998. The AHS subjects for this study are the high-dose (1 Gy or more) and control (less than 0.005 Gy) groups; these total roughly 3,500 in Hiroshima and Nagasaki. About 500 samples that overlap with the F_1 study have been immortalized and are being stored at the Department of Genetics. To date, the immortalization of lymphocytes from Hiroshima subjects is nearing completion. All 1,873 Hiroshima subjects in the targeted subsets have had lymphocytes immortalized. We began immortalizing lymphocytes from Nagasaki AHS participants in October 2000, and lymphocytes from 830 Nagasaki AHS participants have been successfully transformed. In addition we have started EBV-transformation of cryopreserved mononuclear cells from individuals whose other DNA sources (such as paper discs adsorbed with blood) are not currently available due to various reasons, including death or illness.

RP 3-87 Cellular immune function and its relationship to *in vitro* T-lymphocyte radiosensitivity and MN blood group locus mutation frequency in A-bomb survivors: Precursor frequency analysis of mitogen- and antigen-responsive blood lymphocytes (See also RP 1-93)

Kusunoki Y (R), Yoshida K (R), Hamasaki K (G), Hayashi T (R), Imai K (R), Cologne JB (S), Nakachi K

This study aims to evaluate T-cell functions and analyze their relationships to individual sensitivity among A-bomb survivors relative to radiation-induced genetic damage. We analyzed frequencies of T cells that can react to a given stimulus *in vitro* and measured percentages of various lymphocyte subsets in the peripheral blood. Radiation sensitivity was assessed among individuals, using *in vitro* radiosensitivity of T lymphocytes along with T-cell receptor (*TCR*), and glycophorin-A (*GPA*) mutation frequencies (Mf) *in vivo*. Our results indicate that A-bomb radiation has led to a decrease in CD4 T-cell fractions as well as a decrease in frequencies of the T cells that are capable of producing IL-2; conversely, B-cell percentages appear to have increased somewhat. We also showed that *TCR* mutation assay can be used as a sensitive indicator of recent radiation exposure but not as a radiosensitivity biomarker in A-bomb survivors. In contrast, erythrocyte *GPA* Mf increased with radiation dose in A-bomb survivors, and a follow-up study showed that the slope of the dose-response curve was significantly higher in the cancer group than in the cancer-free group among Hiroshima subjects, suggesting that the mutability of somatic genes of irradiated Hiroshima survivors might be associated with cancer susceptibility. On the basis of the assumption that this association in part involves possible difference(s) in genetic background, we initiated association analyses between genotypes of DNA repair genes and *GPA* Mf. Preliminary results suggest that there is an association between the dose-response curve of *GPA* Mf among survivors and p53-binding protein 1 (*P53BP1*) gene polymorphism, but not between the dose-response curve and *ATM* or *NBS1* gene polymorphisms. We have also established flow cytometry systems for measuring radiation-induced phosphorylated histone H2AX (γH2AX) in cultured human T cells, and for measuring micronucleated reticulocyte frequencies in stored red blood cell samples to better evaluate individual sensitivity to radiation-induced genetic damage and instability. With the use of those radiosensitivity biomarkers, we will further investigate relationships between polymorphisms of repair-related genes and *in vitro* lymphocyte radiosensitivity or *in vivo* somatic mutation frequency among A-bomb survivors.

Immunology Studies Publications

RERF Reports (RR)

♦ Kyoizumi S, Yamaoka M, Kubo Y, Hamasaki K, Hayashi T, Nakachi K, Kasagi F, Kusunoki Y: Memory CD4 T-cell subsets discriminated by CD43 expression level in A-bomb survivors. International Journal of Radiation Biology 2010 (January); 86(1):56–62.

 (RR 11-07)

[Abstract] ***Purpose.*** Our previous study showed that radiation exposure reduced the diversity of repertoires of memory thymus-derived cells (T cells) with cluster of differentiation (CD)- 4 among atomic-bomb (A-bomb) survivors. To evaluate the maintenance of T-cell memory within A-bomb survivors 60 years after radiation exposure, we examined functionally distinct memory CD4 T-cell subsets in the peripheral blood lymphocytes of the survivors. ***Methods.*** Three functionally different subsets of memory CD4 T cells were identified by differential CD43 expression levels and measured using flow cytometry. These subsets consist of functionally mature memory cells, cells weakly responsive to antigenic stimulation, and those cells functionally anergic and prone to spontaneous apoptosis. ***Results.*** The percentages of these subsets within the peripheral blood CD4 T-cell pool all significantly increased with age. Percentages of functionally weak and anergic subsets were also found to increase with radiation dose, fitting to a log linear model. Within the memory CD4 T-cell pool, however, there was an inverse association between radiation dose and the percentage of functionally mature memory cells. ***Conclusions.*** These results suggest that the steady state of T cell memory, which is regulated by cell activation and/or cell survival processes in subsets, may have been perturbed by prior radiation exposure among A-bomb survivors.

♦ Yoshida K, Nakachi K, Imai K, Cologne JB, Niwa Y, Kusunoki Y, Hayashi T: Lung cancer susceptibility among atomic bomb survivors in relation to CA repeat number polymorphism of *epidermal growth factor receptor* gene and radiation dose. Carcinogenesis 2009 (December); 30(12):2037–41.

 (RR 10-09)

[Abstract] Lung cancer is a leading cause of cancer death worldwide. Prevention could be improved by identifying susceptible individuals as well as improving understanding of interactions between genes and etiological environmental agents, including radiation exposure. The epidermal growth factor receptor (EGFR)-signaling pathway, regulating cellular radiation sensitivity, is an oncogenic cascade involved in lung cancer, especially adenocarcinoma. The cytosine adenine (CA) repeat number polymorphism in the first intron of *EGFR* has been shown to be inversely correlated with EGFR

production. It is hypothesized that CA repeat number may modulate individual susceptibility to lung cancer. Thus, we carried out a case-cohort study within the Japanese atomic bomb (A-bomb) survivor cohort to evaluate a possible association of CA repeat polymorphism with lung cancer risk in radiation-exposed or negligibly exposed (<5 mGy) A-bomb survivors. First, by dividing study subjects into *Short* and *Long* genotypes, defined as the summed CA repeat number of two alleles ≤37 and ≥38, respectively, we found that the *Short* genotype was significantly associated with an increased risk of lung cancer, specifically adenocarcinoma, among negligibly exposed subjects. Next, we found that prior radiation exposure significantly enhanced lung cancer risk of survivors with the *Long* genotype, whereas the risk for the *Short* genotype did not show any significant increase with radiation dose, resulting in indistinguishable risks between these genotypes at a high radiation dose. Our findings imply that the EGFR pathway plays a crucial role in assessing individual susceptibility to lung adenocarcinoma in relation to radiation exposure.

Other Journal Publication

♦ Hamasaki K, Kusunoki Y, Nakashima E, Takahashi N, Nakachi K, Nakamura N, Kodama Y: Genomic instability in clonally expanded T-lymphocytes from A-bomb survivors in vitro. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2009 (December); 44(4):396–406. (Japanese) (related to *Cytogenetics Studies*)

Manuscripts in Press

⌘ Kusunoki Y, Hamasaki K, Koyama K, Imai K, Hayashi T, Martin PJ, Nakachi K: Increased DNA damage in hematopoietic cells of mice with graft-versus-host disease. Mutation Research.

⌘ Kusunoki Y, Yamaoka M, Kubo Y, Hayashi T, Kasagi F, Douple EB, Nakachi K: T-cell immunosenescence and inflammatory response in atomic-bomb survivors. Radiation Research.

⌘ Mikamoto T, Toyoshima M, Xi Y, Honda H, Hamasaki K, Kusunoki Y, Kamiya K: The effects of overexpressed translesion DNA synthesis polymerase Rev1 on mutagenesis induced by ionizing radiation. Hiroshima Igaku [Journal of the Hiroshima Medical Association] (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

⌘ Toyoshima M, Xi Y, Mikamoto T, Watanabe H, Masuda Y, Honda H, Hamasaki K, Kusunoki Y, Kamiya K: Role of Rev1 in radiation-induced tumorigenesis. Hiroshima Igaku [Journal of the Hiroshima Medical Association] (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

Immunology Studies Oral Presentations

❖ Hayashi T, Morishita Y, Nagamura H, Maki M, Kusunoki Y, Yoshida K, Nakachi K. *IL-18* gene polymorphisms and colorectal cancer risk among atomic-bomb survivors. The 100th Annual Meeting of the American Association for Cancer Research (AACR), 18–22 April 2009, Denver, Colorado, USA

❖ Kusunoki Y. T-cell immunity and inflammatory response among atomic-bomb survivors. Late Health Effects of Ionizing Radiation, 4–6 May 2009, Washington DC, USA

❖ Kusunoki Y, Yoshida K, Hayashi T, Nakachi K. Increases in the percentages of CD43-low memory and CD25+/CD127- regulatory T cells in the CD4 T-cell populations among A-bomb survivors. The 5th Kyoto T Cell Conference (KTCC) 2009 International Workshop on T Lymphocytes, 1–4 June 2009, Kyoto

❖ Ohishi W, Hayashi T, Kusunoki Y, Nakashima E, Fujiwara S, Chayama K. Influence of *HLA-DRB1* Allele on the clearance and persistence of hepatitis C virus. The 45th Annual Meeting of the Japan Society of Hepatology, 4–5 June 2009, Kobe (related to *Special Clinical Studies*)

❖ Hayashi T, Morishita Y, Maki M, Sasaki K, Nagamura H, Sora M, Imai K, Yoshida K, Kusunoki Y, Nakachi K. Molecular epidemiology study of sensitivity to colorectal cancer in atomic-bomb survivor cohort. The Joint Meeting for Cancer Prevention 2009 Aichi, 16–17 June 2009, Nagoya

❖ Imai K, Hayashi T, Nakachi K. Molecular epidemiology of lifestyle-related diseases in relation to biological effects of cigarette smoking, using the data from a general population cohort study. FY2008 Research Meeting of the Smoking Research Foundation, 16 July 2009, Tokyo

❖ Hayashi T, Ohishi W, Imai K, Yoshida K, Hayashi I, Fujiwara S, Kusunoki Y, Nakachi K. Effects of inflammation-related gene polymorphisms and atomic-bomb radiation exposure on risks of stomach and liver cancers. The 16th Annual Meeting of the Japanese Society of Immunotoxicology, 27–28 August 2009, Asahikawa

❖ Hayashi T, Morishita Y, Nagamura H, Maki M, Hayashi I, Yoshida K, Kusunoki Y, Nakachi K. Effects of inflammation-related gene polymorphisms and atomic-bomb radiation exposure on risks of colorectal cancer. The 18th Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics, 25–27 September 2009, Nagoya

❖ Hayashi T, Kusunoki Y, Yoshida K, Imai K, Nakachi K. Colorectal cancer risks of atomic-bomb survivors in relation to *IL-18* gene polymorphisms and prior radiation exposure. The 68th Annual Meeting of the Japanese Cancer Association, 1–3 October 2009, Yokohama

❖ Hayashi T, Ohishi W, Morishita Y, Maki M, Sasaki K, Nagamura H, Sora M, Imai K, Yoshida K, Fujiwara S, Kusunoki Y, Nakachi K. Effects of *IL-10* gene polymorphisms and atomic-bomb radiation exposure on risks of stomach and liver cancers. The 55th Annual Meeting of the Radiation Research Society, 3–7 October 2009, Savannah, Georgia, USA

❖ Yoshida K, Hayashi T, Imai K, Kusunoki Y, Nakachi K. Impact of *ATM*, *ATR*, and *NBS1* genetic polymorphisms on radiation-associated cancer risks in atomic-bomb survivors. Keystone Symposia on Molecular and Cellular Biology: Telomere Biology and DNA Repair, 9–14 October 2009, Ashmore, Australia

❖ Hamasaki K, Imai K, Koyama K, Hayashi T, Nakachi K, Kusunoki Y. Inflammation and genomic instability in murine hematopoietic system. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Kusunoki Y. Immunological alterations in aging A-bomb survivors. 2009 Radiation Effects Association Lecture Meeting: Relationship between Radiation Exposure and

Cancer, 24 November 2009, Tokyo

❖ Kusunoki Y, Yoshida K, Ohishi W, Hayashi T. *NKG2D* genotypes associated with cell surface expression levels in human peripheral blood CD8T and NK cell populations. The 39th Annual Meeting of the Japanese Society for Immunology, 2–4 December 2009, Osaka

❖ Yoshida K, Ohishi W, Chayama K, Kusunoki Y, Hayashi T. Effects of *NKG2D* polymorphism on hepatitis-C virus infection. The 39th Annual Meeting of the Japanese Society for Immunology, 2–4 December 2009, Osaka (related to *Special Clinical Studies*)

Research Protocols 3-10, 2-10, 6-08, 3-05, 2-05, 1-05, 8-02, 5-00, 3-00, 2-99, 9-92, 5-92, 3-89, 4-85, A1-10, A5-09, A14-08, A13-08, A10-08, A8-08, A4-08

Special Clinical Studies

RP 3-10 Ophthalmologic follow-up study in atomic-bomb survivors (Addendum to RP 3-00)

Neriishi K (CH), Yokoyama T, Takamatsu M, Kumagami T, Uematsu M, Tsuiki E, Minamoto A, Kiuchi Y, Kitaoka T, Nakashima E (S), Hida A (CN), Fujiwara S (CH), Akahoshi M (CN)

This addendum proposes to investigate several unanswered questions in the previous ophthalmologic study based on RP 3-00. The ophthalmologic study of 837 A-bomb survivors based on RP 3-00 had revealed a statistically significant dose response with posterior subcapsular and cortical cataracts, and suggested a low dose threshold. This addendum proposes to investigate additional questions, specifically (1) whether or not radiation-induced cataracts progress with time and (2) whether or not there is a dose response when cataracts are assessed by a radiation-specific classification system (the Merriam-Focht method).

Ten years will have passed by June 2010 since the conduct of the RP 3-00 study; stored digital lens images obtained in that study will be compared with those obtained in the proposed study. Ophthalmological examinations on AHS participants will be conducted by ophthalmologists. The methods will be essentially identical to those of the previous study. In particular, (1) subjects will be those who were age 13 or less at the time of the bombs, (2) the Lens Opacity Classification System II (LOCS II) and the Merriam-Focht method will be used for grading. More than 700 AHS subjects under age 13 who participated in the previous study and an additional 300 or more eligible new participants not previously examined are expected to visit RERF during 2010–2012. The statistical power of this study is calculated as more than 90% for the radiation-associated prevalence of cataract cases.

RP 2-10 Evaluation of retinal arteriolosclerosis and age-related macular degeneration using stored retina images with standardized measurements in relation to glaucoma development in atomic bomb survivors and to association with aortic arteriosclerosis (Addendum to RP 1-05)

Neriishi K (CH), Yanagi M, Kawasaki R, Takahashi I (CH), Nakashima E (S), Hsu WL (S), Yokoyama T, Takamatsu M, Kinoshita H, Tsuiki E, Uematsu M, Kumagami T, Kiuchi Y, Kitaoka T, Fujiwara S (CH), Hida A (CN), Akahoshi M (CN)

The preliminary analysis of glaucoma study (RP 1-05) indicated that normal tension glaucoma prevalence is significantly associated with A-bomb radiation, and retinal arteriolosclerosis is reportedly associated with normal tension glaucoma as a causal factor. We plan an evaluation based on standardized retinal measurements of stored retina images. We will investigate if retinal arteriolosclerosis is involved as an intermediate risk factor in radiation-associated glaucoma. Subjects for the association analysis concerning aortic arteriosclerosis are 2,722 persons who underwent screening examinations, including retinal images, for glaucoma during 2006–2008, according to glaucoma study

(RP 1-05). Among them, 1,598 persons with complete data including known radiation dose are subjects for a causal pathway analysis concerning glaucoma.

A semi-automated computer program will measure and calculate the average width of retina vessels and the average diameters will be estimated for arteries and veins. Age-related macular degeneration will be assessed with a protocol and grading scale using by other ocular studies. Graders who are trained at the Melbourne center to maintain adequate intra- and inter-rater agreement, will assess the photographs for signs of age-related macular degeneration in masked fashion. This study would provide evidence regarding a possible mechanism for radiation associated glaucoma.

We will also evaluate the associations of retinal arteriolosclerosis and age-related macular degeneration with aortic arteriosclerosis, as assessed by the augmentation index (AI), brachial ankle pulse wave velocity (baPWV), ankle brachial index (ABI), toe brachial index (TBI), intima media wall thickness (IMT), and Framingham risk scores (FRS).

RP 6-08 Liver stiffness study using elastometer in Hiroshima atomic-bomb survivors

Ohishi W (CH), Tatsukawa Y (CH), Fujiwara S (CH), Hsu WL (S), Kohata M, Yamada M (CH), Nishi N (EH), Tsuge M, Chayama K

The purpose of this study is to determine whether A-bomb radiation exposure has increased liver stiffness, which is a marker of liver fibrosis severity. We will examine the relationship between liver stiffness and radiation dose in order to determine whether radiation exposure is involved in increases of chronic hepatitis and liver cirrhosis. Additionally, we will examine whether increased liver fibrosis is involved, through insulin resistance, in the development of atherosclerotic diseases, in order to elucidate mechanisms of radiation effects underlying these diseases. During two examination cycles (four years), we will measure liver stiffness as a marker of liver fibrosis severity with an elastometer and measure blood cytokines related to chronic inflammation and insulin resistance for about 3,800 AHS participants (including the expanded group of younger survivors) in Hiroshima.

This research protocol was approved in October, 2008, and measurements of liver stiffness and blood cytokines for younger A-bomb survivors have been conducted since November 2008. During the period of November, 2008 and March, 2010, we measured liver stiffness with the elastometer for 1,579 survivors. We also measured blood cytokine levels related to chronic inflammation and/or insulin resistance such as TNF-α, IL-6, IP-10, MCP-1, PAI-1, leptin, resistin, IGF-I, and IGFBP-3 for about 1,451 survivors.

RP 3-05 Inflammation and cancer incidence in atomic bomb survivors

Neriishi K (CH), Hsu WL (S), Nakashima E (S), Little MP, Tatsukawa Y (CH), Nishi N (EH), Soda M (EN), Yamada M (CH), Fujiwara S (CH), Cologne JB (S), Akahoshi M (CN)

Our objective is to investigate the relationship between inflammatory biomarkers and cancer incidence in Adult Health Study (AHS) participants. Experimental and epidemiological studies report a relationship between inflammation and cancer. Because A-bomb survivors have radiation dose-dependent increases of inflammatory biomarkers, we are investigating the relationship between the biomarkers and cancer incidence among 12,870 Adult Health Study participants followed from 1965 to 1999. We will examine white blood cell (WBC) counts (measured since 1958), erythrocyte sedimentation rate (since 1958), alpha 1 and alpha 2 globulin (since 1985), and sialic acid (1988–1992) as parameters in relation to cancer incidence data for 1965–1999 from the Hiroshima and Nagasaki tumor registries. To analyze the data, we plan to apply principal component analysis, growth curve models, and the Cox regression model as preliminary steps. Based on the causal association of radiation, inflammation, and solid cancer incidence, possible causal models (structural equations model or joint model) will be investigated. In addition, a quasi-mechanistic bystander effect model (indirect effect model) will be used to examine the data. One manuscript about longitudinal trends in white blood cell counts was completed. One or more additional manuscripts on causal models for radiation, inflammation, and cancer incidence will be prepared once the analyses are completed.

A manuscript on longitudinal trends in leukocyte counts was accepted by *Journal of Radiation Research* in March 2010. A joint model to estimate causal associations among radiation, inflammation, and solid cancer incidence was specified and analyzed. Three potential types of intermediate variables are being considered: (1) average leukocyte count, (2) longitudinal trends of leukocyte count, and (3) estimating a latent inflammatory factor using multiple indicators. A preliminary result of a joint model using persons' average WBC counts as a mediating factor shows that there is a significant causal association of radiation, average WBC count, and solid cancer incidence. The proportion of the mediating effect is about 8% of the total radiation effect on cancer.

RP 2-05 Could genetic factors cause population bias among proximal A-bomb survivors?—A test of whether the same genetic factors are risk factors for high inflammatory status and myocardial infarction among A-bomb survivors 40–50 years later

Fujiwara S (CH), Suzuki G, Ohishi W (CH), Akahoshi M (CN), Cologne JB (S)

The primary goal of this study is to evaluate a set of underlying genetic factors associated with survival among atomic-bomb survivors who probably suffered from radiation injuries, burns and early infections, in order to determine if these factors also increased the risk of chronic inflammation and myocardial infarction. Subjects are all the 1,100 individuals who participated in the first cycle of clinical Adult Health Study examinations and who were exposed when young (<30 years of age at the time of the bombings) to at least 1 Gy of radiation, and 1,100 of their sex-, age-, and city-matched <5 mGy exposed controls. We are testing known genetic polymorphisms within an array of genes recognized to be responsive to external stress based on the hypotheses that (1) similar genetic polymorphisms control the magnitude of inflammatory responses to many lifestyle-associated stresses; and that (2) these same polymorphisms

serve as risk factors for cardiovascular disease. In this regard, a candidate genetic haplotype, namely the haplotype block comprising the *LTA*, *NFKBIL1*, and *BAT1* genes in the class III major histocompatibility complex (MHC) region, has been selected.

Such an effect could have led to a "population bias" in cardiovascular disease risk, especially in the early decades after the A-bomb exposure. Specific genetic polymorphic loci will be examined, including *LTA* single nucleotide polymorphisms (SNP), representative of the haplotype-block encompassing the *LTA*, *NFKBIL1*, and *BAT1* genes, and a *TLR2* deletional polymorphism in exon 1.

A total of 2,274 study subjects were selected for this study, and stored blood samples of 1,928 subjects from whom we have obtained an informed consent for genetic studies are being used. We established a new method using a TaqMan probe to analyze *LTA* and *TRL2* gene polymorphisms. Genomic DNA has been extracted from blood samples of 1,303 subjects.

RP 1-05 Glaucoma study in atomic bomb survivors

Kiuchi Y, Yokoyama T, Uematsu M, Tsuiki E, Kitaoka T, Nakashima E (S), Neriishi K (CH), Hida A (CN), Fujiwara S (CH), Akahoshi M (CN)

It is well known clinically that acute, high radiation exposure can induce glaucoma. However, the relationship between glaucoma and radiation exposure within the general population and with moderate doses, such as the AHS cohort is unclear. Two earlier studies yielded inconsistent findings: In one study, a significant negative association between the incidence of glaucoma and radiation dose was found; while in a second study, related to the assessment of cataract formation, no relationship between radiation dose and glaucoma-associated findings (i.e., optic nerve papilla atrophy and intraocular pressure) was apparent. Neither study used sufficiently sophisticated measurements to draw firm conclusions about the actual relationship.

Accordingly, a new, more comprehensive study was initiated in order to definitively determine the relationship between radiation dose and prevalence of glaucoma. Screening examinations, including retinal images, for glaucoma were conducted during 2006–2008. Of 3,546 eligible AHS participants, 2,613 (73.7%) participated in the study, and among 1,589 subjects with known radiation dose (mean age 74.3, range 61 to 97 years) we detected 284 (17.9%) cases of overall glaucoma, 36 (2.3%) cases of primary open angle glaucoma with intraocular pressure levels greater than 21 mmHg, 226 (14.2%) cases of normal tension glaucoma, and 25 (1.6%) cases of primary angle closure glaucoma. Binary regression using the generalized estimating equation method, with adjustment for sex, age, city, cataract surgery, and diabetes mellitus, revealed odds ratios per Gy of 1.31 (95% confidence interval: 1.11–1.53, $p = 0.001$) in the case of normal tension glaucoma. Thus, the results indicate that prevalence of normal tension glaucoma increases with increasing A-bomb radiation dose and a manuscript will soon be submitted to the internal review committee.

RP 8-02 Ophthalmologic study of children of atomic bomb survivors (Addendum to RP 1-02)

Minamoto A, Yokoyama T, Mishima HK, Kitaoka T, Nakashima E (S), Neriishi K (CH), Hida A (CN), Fujiwara S (CH), Akahoshi M (CN)

The study is designed to investigate on a quantitative basis the opacity of eye lenses and retinal arteriolosclerosis of the offspring of A-bomb survivors, and to look into the relationship of this value with several risk factors. We will also study whether or not the incidence of age-related cataracts and congenital cataracts increases due to A-bomb exposure in the parents. The digital images were computerized and stored.

Cataract is a multifactorial disease that stems from a combination of environmental and genetic factors. Animal experiments have suggested the possibility of an increase in the incidence of congenital cataracts in relation to radiation exposure. However, since the expected number of cases is small, we probably will study it qualitatively rather than quantitatively. The study is quantitatively investigating lens opacity and retinal arteriolosclerosis in the offspring of A-bomb survivors who are at least 50 years of age at the time of examination. It is examining the association of lens opacity with both multifactorial diseases and parental radiation doses. The examinations were completed by September 2006. During the study period, inter- and intra-observer reproducibility standardization between two cities was conducted every six months, and 2,517 F_1 individuals in total underwent the ophthalmologic examination. Data have now been compiled and cleaned up, and preliminary results indicated that there was no association between parental radiation and any types of cataract in the offspring. We will conduct further analyses to look into the relationship of the cataracts with several risk factors.

RP 5-00 The prevalence, incidence, and prognosis of the Brugada type electrocardiogram: A population-based study of four decades

Haruta D, Matsuo K, Akahoshi M (CN), Nakashima E (S), Suyama A (EN), Seto S

To clarify the incidence and prognosis of the Brugada type electrocardiogram (ECG) and examine the relationship between Brugada type ECG and sex hormones.

The incidence and prognosis of the Brugada syndrome, a new clinical entity causing sudden death due to ventricular fibrillation (VF), has not been fully elucidated. Although Brugada syndrome, which is linked to mutations of the *SCN5A* gene, is inherited with equal frequency by men and women, most of the reported cases have been adult men.

We identify the Brugada type ECG cases by reviewing all the ECG recordings from 1958 to 1999 in 4,788 Nagasaki AHS subjects who were under age 50 in 1958 to calculate the incidence. We ascertain the sudden death cases from all deceased subjects to evaluate the prognosis of Brugada type ECG cases. We also evaluate the association between Brugada type ECG and prostatic cancer, which is related to testosterone.

We found an association between the Brugada type ECG and prostatic cancer. In 2 surgical castration and one hormonal castration cases, the Brugada type ECG disappeared after castration; leading to a new therapeutic strategy

to prevent sudden death.

The Incidence of the Brugada type ECG was 31.4 persons/100,000 person-years in males, 9 times higher than in females. Brugada type ECG cases had a higher risk of sudden death (RR = 52, 95% CI: 23–128) than controls. Brugada type ECG cases had a higher risk for prostate cancer (RR = 5, 95% CI: 2–15).

RP 3-00 Ophthalmologic study of atomic bomb survivors

Minamoto A, Yokoyama T, Mishima HK, Kitaoka T, Nakashima E (S), Neriishi K (CH), Hida A (CN), Fujiwara S (CH), Akahoshi M (CN)

The goal of this study is to evaluate the prevalence of radiation cataracts within two study subject groups, namely (1) a select group of survivors who were relatively young (13 years old or younger) at the time of the atomic bombing, but had not been previously given ophthalmic examinations; and (2) a larger group that had been evaluated previously, but by older methods. For both groups, dose-response analyses were conducted for posterior subcapsular axial opacities (Figure) and polychromatic changes and for peripheral opacities using a standard grading system, while adjusting for a variety of potential confounding factors. Further, all digital computer images of radiation cataracts were stored for future assessments. A total of 883 persons underwent ophthalmologic examinations in Hiroshima and Nagasaki, from which three papers were published (Minamoto et al., *International Journal of Radiation Biology* 2004; 80:339–45, Nakashima et al., *Health Physics* 2006; 90[2]:154–60, and Nakashima et al., *Annals of the Institute of Statistical Mathematics* 2008; 60[3]:465–82). An analysis of the prevalence of severe cataract cases with surgical lens removal was also published (Neriishi et al., *Radiation Research* 2007; 168:404–8).

Stored lens images collected during 2000–2002 were used to conduct a re-evaluation with the Merriam-Focht cataract scoring method, a radiation-specific classification system used in numerous studies, such as among Chernobyl clean-up workers. The preliminary results of an opacity re-evaluation with the Merriam-Focht method indicate that the dose responses for the two major research cohorts, A-bomb survivors and Chernobyl clean-up workers, are almost identical. The results were presented at an RERF colloquium and a draft paper has been prepared.

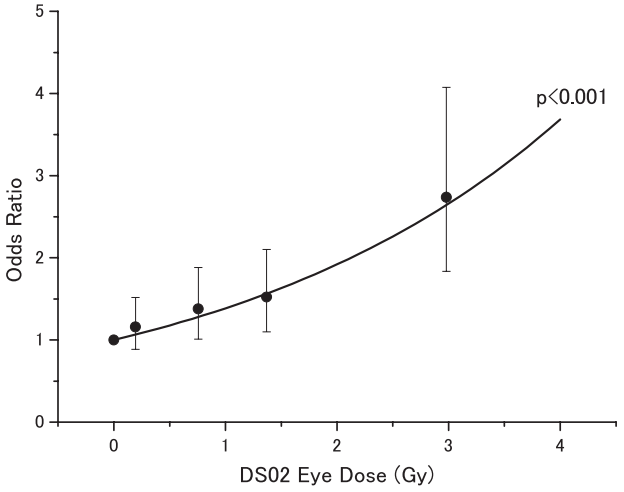


Figure. The main-effect model dose-response curve in regression analysis after adjustingfor city, sex, age at the time of the bombings, and diabetes mellitus in A-bomb survivors who had undergone lens removal surgery (OR at 1 Gy, 1.39; CI, 1.24–1.55).

The results regarding cataract prevalence during 2000–2002 and cataract surgery incidence were presented at the international Radiation Cataractogenesis Workshop held in March 2009 at RERF, which provided strong consensual evidence of a low radiation dose-effect threshold for cataracts. A Workshop summary, including the current results, is to be published in the May 2010 issue of *Radiation Research*.

RP 2-99 Thyroid diseases in Hiroshima and Nagasaki atomic bomb survivors

Imaizumi M (CN), Usa T, Tominaga T, Akahoshi M (CN), Soda M (EN), Neriishi K (CH), Fujiwara S (CH), Yamada M (CH), Kodama K (CS), Nakashima E (S), Shibata Y, Okubo M, Ashizawa K, Sera N (CN), Eguchi K

The objectives of the current thyroid disease study in Hiroshima and Nagasaki AHS cohort are to investigate whether there are positive associations between radiation dose and thyroid nodules, autoimmune thyroid diseases and thyroid dysfunction, and further, to examine whether thyroid cancers frequently develop among subjects with thyroid nodules detected in an earlier study (1984–87).

We found that malignant thyroid tumor, benign nodules and cysts were increased with radiation dose and the relationships were more significant in those exposed at younger ages. On the other hand, autoimmune hypothyroidism and Graves' disease were not associated with radiation dose (Imaizumi et al., *JAMA* 2006; 295[9]:1011–22). No significant dose-responses for thyroid diseases were observed among those exposed *in utero*, although the risk estimates were similar to those with juvenile exposure and the null results may reflect limited statistical power (Imaizumi et al., *Journal of Clinical Endocrinology and Metabolism* 2008; 93:1641–8) (Table). We more frequently detected cancer in subjects with solid thyroid nodules than in nodule-free controls (Imaizumi et al., *Journal of Clinical Endocrinology and Metabolism* 2005; 90:5009–14), suggesting that a thyroid nodule is a risk factor for subsequent thyroid cancer.

Table. Odds ratios (ORs) of all solid thyroid nodules by mode of exposure

	No. (%)	OR at 1 Gy (95% CI)	*P*
In utero exposure	35/319 (11.0)	2.91 (0.53, 12.18)	0.20
Childhood exposure	63/437 (14.4)	2.65 (1.96, 3.65)	<0.001
Combined*	98/756 (13.0)	2.66 (1.97, 3.63)	<0.001

* Combined analysis of *in utero* exposure and childhood exposure.

We are now conducting thyroid examinations in the newly expanded cohort of AHS subjects to study the effects of low dose radiation on thyroid diseases exposed at younger age based on RP 3-07.

RP 9-92 Study of liver diseases in the Adult Health Study sample: Relationship between radiation dose and infection by hepatitis B and C viruses

Ohishi W (CH), Fujiwara S (CH), Cologne JB (S), Cullings HM (S), Nakashima E (S), Kusunoki Y (R), Hayashi T (R), Yoshida K (R), Akahoshi M (CN), Chayama K

The hypothesis behind this study is that ionizing radiation may increase the incidence of hepatocellular carcinoma either by increasing the rates of hepatitis B or C virus infection or by facilitating disease progression after hepatitis virus infection. The purposes of this study are to investigate the associations of radiation with (1) rates of hepatitis C virus (HCV) infection, (2) hepatitis B virus (HBV) activity (hepatitis B e-antigen: HBeAg) in HBV carriers, and (3) HBeAg and hepatitis B surface antigen (HBsAg) seroconversion rates among HBV carriers. We will follow up HCV antibody-positive subjects and HBV carriers in an attempt to improve understanding of the natural history of HCV and HBV-related liver disorders. It is expected that the data will provide the foundation essential for quantifying the risk of liver disorders associated with HCV and HBV infection among A-bomb survivors.

Our previous studies demonstrated that the prevalence of HBsAg increased with radiation dose among the AHS. The percent of subjects who were unable to clear the virus increased significantly with radiation dose among those who had received blood transfusions. No relationship was found between radiation dose and the prevalence of anti-HCV, but the radiation dose response for chronic liver disease among anti-HCV-positive subjects was suggestively greater than that among anti-HCV-negative subjects.

We have assessed the effects of immunogenetic background (*HLA-DRB1* and/or *NKG2D* polymorphisms) and radiation dose on the course following HCV infection (RP 4-04) in collaboration with the Department of Radiobiology/Molecular Epidemiology. We have also conducted a longitudinal analysis of the development of HCC by status of HBV/HCV infection measured in 1993–1995 in relation to radiation exposure among the AHS. Preliminary analyses suggested that *HLA-DRB1* and/or *NKG2D* polymorphisms affect the clearance and/or persistence following HCV infection, and that an *NKG2D*-mediated immune response is involved in the mechanisms underlying HCV clearance.

RP 5-92 Study on senile dementia among the Adult Health Study subjects

Yamada M (CH), Fujiwara S (CH), Mimori Y, Sasaki H, Akahoshi M (CN), Nakamura S, Kasagi F (EH), White LR

In this study, we examined the effects of radiation exposure on cognitive function, the prevalence and incidence of dementia, and other age-related physiologic variables such as reaction time at older ages among adult survivors in the AHS.

A wide spectrum of radiation effects on the central nervous system have been well documented in RERF studies, especially for individuals who were exposed prenatally or during childhood. This study investigates the hypothesis that the effects of ionizing radiation on the mature central nervous system may be manifested as accelerated neurological aging. In the late 1980s, a collaborative study of dementia using standardized procedures to compare Japanese Americans living in Seattle and Honolulu with the AHS cohort was initiated to identify whether the prevalence, incidence, and causes of dementia were the same across cultures (the Ni-Hon-Sea study).

Study subjects were survivors exposed at ≥13 years of age. We evaluated cognitive performance for about 3,113 subjects in Hiroshima and Nagasaki with the Cognitive Abilities Screening Instrument (CASI) during the period 1992–1998. The prevalence of dementia and its subtypes was assessed among 2,648 Hiroshima AHS subjects aged 60 years or older at baseline examination (1992–1996). Dementia prevalence was observed, and 2,286 dementia-free subjects were followed up to assess dementia incidence.

Manuscripts regarding radiation effects and other risk factors on dementia incidence have been published (Yamada et al., *Journal of the Neurological Sciences* 2009; 281:11–4 and Yamada et al., *Journal of the Neurological Sciences* 2009; 283:57–61) (Table). Although no association was found between previous radiation exposure and cognitive impairment and/or development of dementia among survivors exposed at ≥13 years of age, survivors exposed <13 years of age may well be more vulnerable. We are considering potential instruments for assessing cognitive and other psychoneurological function among the younger and *in utero* survivors. A new research protocol is being prepared.

Table. Radiation effects on dementia incidence (Results of Poisson regression analysis)

	Hazard ratio	95% CI	*p*-value
All dementia			
dose 1	0.82	0.59–1.14	0.238
dose 2	0.94	0.65–1.33	>0.5
Probable AD			
dose 1	0.64	0.37–1.09	0.105
dose 2	0.94	0.54–1.62	>0.5
Possible AD			
dose 1	0.88	0.45–1.09	>0.5
dose 2	0.87	0.40–1.81	>0.5
Probable VaD			
dose 1	0.84	0.37–1.84	>0.5
dose 2	0.77	0.32–1.77	>0.5

Dose 1: 5–499 mGy group vs <5 mGy group
Dose 2: ≥500 mGy vs <5 mGy group
CI: confidence interval, AD: Alzheimer's disease, VaD: vascular dementia
Model is adjusted for age, $(age)^2$, education, BMI, smoking, drinking, menopausal age, and history of hypertension, diabetes, and stroke.

RP 3-89 Osteoporosis in Hiroshima atomic-bomb survivors

Fujiwara S (CH), Masunari N (CH), Furukawa K (S), Kasagi F (EH), Fukunaga M, Orimo H

The overall goal of this study is to determine the relationship between ionizing radiation and the prevalence and severity of osteoporosis as a potential, long-term health consequence of prior radiation exposure. We anticipate that this study will provide comprehensive answers to questions

related to radiation exposure and its potential to predispose aging individuals to osteoporosis and related health risks. Our working hypothesis is that acute ionizing radiation might accelerate the aging process as manifested by increased osteoporosis. To date, preliminary analyses of bone mineral density (BMD) in long-term atomic-bomb survivors do not suggest radiation exposure-related changes in BMD, even after adjusting for age, weight, and age at menopause. We are utilizing accumulated data on BMD and fracture for national and international collaborative studies.

A more in-depth follow-up study is planned for all Adult Health Study (AHS) participants in Hiroshima and will examine the relationship between radiation exposure and the development of osteoporosis as an index of aging. All the persons who participate in the AHS examination will continue to be evaluated on a periodic basis for BMD by means of bone densitometry, bone metabolic markers (urinary γ-GTP, serum FGF-23, etc.), vertebral compression fractures observed in diagnostic X-ray studies, and a number of other possible factors such as weight, exercise, gynecological history, and medication and diseases.

This osteoporosis study using the AHS population continues to be a leader with respect to international and national collaborations. For instance, in the WHO collaborative study of 11 cohorts in Hiroshima, Europe, Australia, and the USA, we established a program for identification of patients at high risk of osteoporotic fracture, attempting to optimize the sensitivity of assessment so that therapy can be better directed. A paper obtained from this international collaborative study with WHO working group has been published in an international journal (Fujiwara et al., *Osteoporosis International* 2008; 19:429–35). We also are collaborating in several multi-institutional studies of osteoporosis etiology or prevention in Japan. We have been measuring bone biochemical markers (FGF-23, γ-GTP, NTx, CTx) to determine if these markers could predict future fracture in collaboration with National Institute for Longevity Sciences. Invited review papers related to osteoporosis and fractures have been published (Masunari et al., *Health-Related Quality of Life*. 2009, pp 1–29 and Masunari et al., *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery* 2010; 4:15–33).

RP 4-85 Incidence and risk factors of coronary heart disease (CHD) in Japanese men living in Japan and Hawaii, 1966–78 (Addendum to Research Plan TR 12-71)

Yamada M (CH), Kodama K (CS), Tatsukawa Y (CH), Shimizu Y (EH), Kasagi F (EH), Sasaki H, Takahashi I (CH), Fujiwara S (CH), Curb JD, Rodriguez B, Yano K

The objective of the study to investigate the relationship between risk factors and the incidence of cardiovascular disease (CVD) among Japanese men living in Japan and in the United States (The NI-HON-SAN project). The epidemiologic methodology developed by this project has been applied for the entire AHS cohort. The methodology has been effective in indicating a weak, but very consistent association between radiation dose and various endpoints of atherosclerosis, including myocardial infarction, stroke, calcification of the aortic arch, retinal arteriosclerosis, isolated systolic hypertension, and abnormal pulse wave velocity.

The cases of CHD (mainly acute myocardial infarction) and cerebrovascular disease are being ascertained through periodic examination, mortality surveillance, autopsy, etc. In order to obtain more detailed information, particularly on acute coronary events, a mail survey for morbidity surveillance has been conducted every six months in the AHS cohort since 1995. Data regarding atherosclerotic endpoints and risk factors has been collected.

An RP to assess atherosclerosis and arterial stiffness using equipment to measure central blood pressure, augmentation index (AI), brachial-ankle pulse wave velocity (baPWV), and ankle brachial index (ABI) in relation to radiation exposure has been approved and will start in April 2010.

This study is helping the cardiovascular working group develop hypotheses regarding low-dose radiation effects on cardiovascular disease. Radiation-induced inflammation is one of the hypotheses. Measurements of some biomarkers for inflammation and oxidative stress have been initiated. A new RP to measure cytokines activating the development of atherosclerotic changes has been developed.

RP-A1-10 Radiosensitivity difference of cataract surgery in A-bomb survivors by polymorphisms of *ATM* and other genes

Neriishi K (CH), Hayashi T (R), Nakashima E (S), Misumi M (S), Nakachi K

An association between ataxia telangiectasia mutated (*ATM*) gene and lens radiosensitivity has been well documented in experimental animals. Worgul et al. reported that atm heterozygous (+/–) mice are more sensitive to radiation-induced cataracts than are their wild-type counterparts. Kleiman et al. reported that Mrad9 and atm haploinsufficiency enhance spontaneous and X-ray-induced cataractogenesis in mice. Since A-bomb survivors demonstrated a significant dose-response relationship in prevalence of cataract surgery with A-bomb radiation, and since genotyping data on *ATM* and other genes are available from another study at RERF (RP 4-04), this protocol aims to investigate an association between polymorphisms of *ATM* and other genes and the dose-dependent prevalence of cataract surgery in A-bomb survivors. Subjects are 5,126 AHS participants who underwent medical examination during 2000–2001. Among them, those who have an information of polymorphism of *ATM* and other genes in the study of RP 4-04 and who agreed to use the information for the study were selected. In 5,126 AHS participants, there were 645 persons with cataract surgery.

The dataset of polymorphisms of *ATM* and other genes will be obtained from the Department of Radiobiology/Molecular Epidemiology and merged with the cataract surgery data. The prevalence of cataract surgery will be compared by radiation dose and polymorphisms of *ATM* and other genes. Two types of analysis are envisioned. One would be based on individual candidate SNPs and the other on haplotypes.

RP-A5-09 Application of causal modeling on radiation, inflammation, and cataract surgery incidence among Adult Health Study population

Kakuma T, Araki Y, Hsu WL (S), Nakashima E (S), Neriishi K (CH)

An RERF study has indicated a significant dose response for the prevalence of cataract surgery in A-bomb survivors. It is hypothesized that the effect of radiation exposure on cataract risk may be mediated through an inflammation process. This study proposes to apply a causal model to address the complex associations of radiation, inflammation, and cataract incidence, i.e., a joint model in which the associations among radiation, inflammation, and incidence of cataract surgery are simultaneously modeled and estimated in the time-to-event analysis for the A-bomb survivors. The RP was approved in fall 2009. Dr. Hsu started by analyzing the data using Mplus, a specialized statistical package for latent variable models. The preliminary results from Mplus indicated significant causal association of radiation, inflammation, and cataract surgery. The proportion of indirect effect, i.e., radiation effect on cataract mediated by inflammation, was about 8%. Further analysis is in progress. Drs. Kakuma and Araki at Kurume University are working on deriving the theoretical framework under the same causal hypothesis and plan to analyze the model in STATA.

RP-A14-08 The incidence and prognostic value of the early repolarization electrocardiogram pattern

Haruta D, Tsuneto A, Nakashima E (S), Akahoshi M (CN)

The early repolarization pattern (ERP) has been considered to be benign, but one recent high-profile clinical study reported its potential arrhythmogenicity, suggesting the possibility that ERP is a cause of idiopathic ventricular fibrillation leading to sudden death.

Of 7,564 subjects (3,374 men and 4,190 women) followed biennially in Nagasaki since 1958, we will investigate all ECG records of 5,976 subjects who have been examined at least once in Nagasaki between 1958 and 2004. We found 650 prevalent ERP cases and 779 incident ERP cases. The incidence of ERP peaked during the fourth decade of life. ERP appeared intermittently on inferior and/or lateral leads. We found 27 and 42 sudden death cases in 1,429 ERP cases and 4,507 control subjects, respectively. The rates of sudden death in ERP cases and control subjects are 1.89% and 0.93%, leading to approximately two times higher rate of sudden death in ERP cases compared to that in control subjects. In this study, we found 7 sudden death cases (17.5%) among 40 Brugada type ECG cases. While the rate of sudden death in ERP cases is approximately one-tenth of that in Brugada type ECG cases, ERP is an important issue of public health, because approximately 32% of subjects exhibit ERP and 36% of sudden death are related to ERP. We are preparing a manuscript to submit.

RP-A13-08 Prognostic significance of ventricular premature contractions (VPCs) in taking consideration of their origins

Haruta D, Nakashima E (S), Fujiwara S (CH), Akahoshi M (CN)

Ventricular premature contractions (VPCs) are common arrythmias in patients with and without structural heart diseases, and the presence of VPC in regular 12-lead ECG recordings is a significant and independent predictor for cardiovascular mortality.

We will extract VPC cases using the ECG database among AHS subjects (4,092 in Hiroshima and 2,642 in Nagasaki) who underwent a regular 12-lead ECG recording in Nagasaki and Hiroshima from January 1990 to December 1993 and classify VPC into three groups according to the morphology of VPC in the precordial lead; (1) Left Bundle Branch Block (LBBB) type that originates from the right ventricle; (2) Right Bundle Branch Block (RBBB) type that originates from the left ventricle, and; (3) unidentified type. Information of deceased cases and cause of death until December 2005 will be used to assess the prognostic significance for cardiovascular mortality between cases with and without VPC. We will characterize the certainty of the VPC diagnosis by whether it was observed at only one or more than one clinic visit. We will conduct Cox proportional hazards analyses to assess the prognostic significance of VPC, frequency of VPC diagnosis, and morphology of VPC for cardiovascular mortality after adjusting for age, sex, and underlying diseases.

RP-A10-08 The association between subclinical thyroid dysfunction and cardiovascular disease and mortality: An individual participant pooled analysis of large international cohort studies

Rodondi N, Gussekloo J, Imaizumi M (CN)

This is an international collaborative study involving nine cohorts in Europe, USA, Australia, and Japan (Nagasaki AHS). The objective of this study is to assess the relationship between subclinical thyroid dysfunction and coronary heart disease and mortality.

There are conflicting data from prospective cohort studies regarding the association between subclinical hypothyroidism and cardiovascular outcomes. These conflicting results might reflect differences in participants' age, gender, thyrotropin (TSH) levels or preexisting cardiovascular disease.

We performed an individual participant data analysis of 41,685 participants (2,621 with subclinical hypothyroidism) in nine prospective cohort studies with 381,647 person-years of follow-up. We examined the risk of coronary heart disease (CHD) and total mortality in all cohorts, and the risk of CHD events in 13,355 participants from six cohorts with such data available. Euthyroidism was defined as a TSH 0.50–4.49 mU/L and subclinical hypothyroidism as a TSH ≥4.5–19.9 mU/L with normal thyroxine concentrations.

Over follow-up, 7,770 participants died (1,715 from CHD), and 3,745 participants had CHD events (among six studies). In age and gender-adjusted analyses, the hazard ratio (HR) for participants with subclinical hypothyroidism compared with euthyroidism was 1.25 (95% confidence interval, 1.00–1.57) for CHD events, 1.14 (0.98–1.34) for CHD mortality, and 1.09 (0.94–1.25) for total mortality. The risk of CHD increased with higher TSH concentrations (p for trend = 0.007), with HRs of 1.95 (1.21–3.15) for CHD events and 1.64 (1.11–2.42) for CHD mortality among those with TSH ≥10 mU/L compared with euthyroid participants.

Results were similar after further adjustment for traditional cardiovascular risk factors. Risks did not significantly differ by age, gender, or preexisting cardiovascular disease. In conclusion, subclinical hypothyroidism is associated with an increased risk of CHD events and CHD mortality in those with higher TSH levels. Papers are currently under review.

RP-A8-08 Incidence and risk factors of fatty liver

Tsuneto A, Nakashima E (S), Akahoshi M (CN)

Since fatty liver predicts ischemic heart disease, the incidence and predictors of fatty liver need examination. This study aims to determine fatty liver incidence and predictive variables.

Using abdominal ultrasonography, we followed biennially during1990–2007 (mean follow-up, 11.6 ± 4.6 years) 1,635 Nagasaki atomic-bomb survivors (606 men) without fatty liver at baseline. We examined potential predictive variables with the Cox proportional hazards model and longitudinal trends with the Wilcoxon rank-sum test.

323 (124 male) new fatty liver cases were diagnosed. The incidence was 19.9/1,000 person-years (22.3 for men, 18.6 for women) and peaked in the sixth decade of life. After controlling for age, sex, smoking, and drinking habits, obesity, low HDL-cholesterol, hypertriglyceridemia, glucose intolerance, and hypertension were predictive of fatty liver. In the multivariate analysis including all variables, obesity, hypertriglyceridemia, and hypertension remained predictive. In fatty liver cases, body mass index and serum triglycerides, but not systolic or diastolic blood pressure, increased significantly and steadily up to the time of the diagnosis. Radiation dose was not associated with fatty liver (RR = 0.92, 95% CI: 0.8–1.1). In summary, obesity, hypertriglyceridemia, and, to a lesser extent, hypertension might serve as predictive variables for fatty liver.

RP-A4-08 Study on the estimability of waist circumference and its application to risk analysis for metabolic syndrome

Nakamura T, Ichimaru S (CN), Ishida N, Soda M (EN), Akahoshi M (CN), Cullings HM (S), Nakashima E (S), Misumi M (S)

Metabolic Syndrome (MS) is a combination of medical risk factors for developing cardiovascular disease and diabetes. World-wide, many different sets of criteria have been proposed for the diagnosis of this syndrome. A common feature of these criteria is that they all utilize abdominal fat accumulation, as estimated by the waist circumference (WC) measured at the level of the umbilicus, as one criterion for the diagnosis of the syndrome. The objective of this research is to examine the projection of estimates of WC obtained from recently measured WC on RERF AHS subjects who came to the clinic in 2005–2006 to AHS subjects who had MS related deaths prior to 2005 when no WC measures were taken. Using the 2005–2006 data, we will obtain theoretically unbiased estimates of WC and their measurement error, for each subject ten years prior to 2005 (when WC was not measured) through an extrapolation procedure that incorporates an analysis of other correlated covariates from the accumulated health examination data at RERF. Then, a proportional hazards model adjusted for the measurement error will be developed. Finally, we will describe how to design a retrospective cohort study for MS using RERF data. Dr. Ishida completed an analysis for her Ph.D. thesis and wrote a paper about the risk of the MS-related causes of death that was published in the *Japanese Journal of Biometrics*. The results of risk estimation for death indicate that, for those of Semi-MS (those with at least two of dyslipidemia, hypertension, and impaired glucose tolerance), the larger the WC, the smaller the risk of MS-related death, contrary to expectations. This requires further investigation.

Special Clinical Studies Publications

RERF Reports (RR)

♦ Kasagi F, Yamada M, Sasaki H, Fujita S: Biologic score and mortality based on a 30-year mortality follow-up: Radiation Effects Research Foundation Adult Health Study. Journals of Gerontology, Series A: Biological Sciences 2009 (August); 64A(8):865–70.

 (RR 2-03)

[Abstract] This study aimed to test whether scored biologic functions can predict individual life expectancies and to investigate the disease-related and time-related differences in evaluated associations. A biologic score was defined as the first principal component score of the five physiological tests. Study participants were 4,871 people aged 35–74 years at baseline examination in 1970–1972 and followed until the end of 1999. We evaluated the prognostic value of the biologic score by Cox proportional hazard analysis. In all age and sex groups, increasing trends of mortality for all diseases by increment of biologic score were observed after adjustment for potential risk factors. The validity of the biologic score was significant throughout the entire study period. Each disease except cancer showed a significant association with biologic score at baseline examination. In conclusion, the biologic score is a valid predictor of life span in this large-scale prospective study of middle-aged and elderly Japanese.

♦ Yamada M, Kasagi F, Mimori Y, Miyachi T, Ohshita T, Sasaki H: Incidence of dementia among atomic-bomb survivors—Radiation Effects Research Foundation Adult Health Study. Journal of the Neurological Sciences 2009 (June); 281(1-2):11–4.

 (RR 12-08)

[Abstract] Radiotherapy has been reported to cause neuropsychological dysfunction. Here we examined whether exposure to atomic bomb radiation affected the incidence of dementia among 2,286 atomic bomb survivors and controls—all members of the Adult Health Study cohort. Study subjects were non-demented and aged ≥60 years at baseline examination and had been exposed in 1945 at ≥13 years of age to a relatively low dose (≤4 Gy), compared with total dose from radiotherapy. Dementia diagnoses were made during biennial health examinations with a two-phase procedure. DSM IV criteria were used for diagnosing dementia, NINCDS-ADRDA for Alzheimer disease, and NINDS-AIREN for vascular disease. To estimate the effect of radiation on the dementia incidence rate, we applied Poisson regression analysis. Incidence

per 1,000 person-years was 16.3 in the <5 mGy group, 17.0 in the 5–499 mGy group, and 15.2 in the ≥500 mGy group. Alzheimer disease was the predominant type of dementia in each dose category. After adjustment for potential risk factors, radiation exposure did not affect the incidence rate of either all dementia or any of its subtypes. No case of dementia had a history of therapeutic cranial irradiation. Although we found no relationship between radiation exposure and the development of dementia among atomic bomb survivors exposed at ≥13 years old in this longitudinal study, effects on increased risk of early death among atomic bomb survivors will be considered.

Other Journal Publications

♦ Fujiwara S: Lifestyle and bone mineral density. Seijinbyo to Seikatsu Shukan-Byo [Journal of Adult Diseases] 2009 (May); 39(5):519–23. (Japanese)

♦ Fujiwara S: "Osteoporosis and compression fracture" Epidemiology of osteoporosis. Rinsho Gazo [Clinical Imagiology] 2009 (August); 25(8):822–7. (Japanese)

♦ Fujiwara S: Advancement in osteoporosis treatment—Definition and evaluation of absolute bone-fracture risk. Naika [Internal Medicine] 2009 (September); 104(3):428–31. (Japanese)

♦ Fujiwara S: Development of WHO FRAX and significance. Orthopaedic Surgery and Traumatology 2009; 52(11):1285–92. (Japanese)

♦ Fujiwara S: Exercises and evaluation of physical activity among the elderly. Kurosawa A, ed. Therapeutic Exercise for Chronic Locomotive Diseases. Tokyo: Kanehara-Shuppan; 2009, pp 268–73. (Japanese)

♦ Ishida N, Ichimaru S, Hida A, Soda M, Nakamura T, Akahoshi M: Risk assessment of metabolic syndrome using transported estimates in retrospective cohort study. Keiryo Seibutsugaku [Japanese Journal of Biometrics] 2009 (October); 30(2):93–104. (Japanese)

♦ Masunari N, Fujiwara S: Impact factors of osteoporosis on health-related quality of life. Hoffman EC, ed. Health-Related Quality of Life. New York: Nova Science Publishers; 2009, pp 1–29.

♦ Yamada M, Mimori Y, Kasagi F, Miyachi T, Ohshita T, Sasaki H: Incidence and risks of dementia in Japanese women: Radiation Effects Research Foundation Adult Health Study. Journal of the Neurological Sciences 2009 (August); 283(1-2):57–61.

Manuscripts in Press

⌘ Ashizawa K, Imaizumi M, Usa T, Tominaga T, Sera N, Hida A, Ejima E, Neriishi K, Soda M, Ichimaru S, Nakashima E, Fujiwara S, Maeda R, Nagataki S, Eguchi K, Akahoshi M: Metabolic cardiovascular disease risk factors and their clustering in subclinical hypothyroidism. Clinical Endocrinology.

⌘ Blakely EA, Kleiman NJ, Neriishi K, Chodick G, Chylack LT, Cucinota FA, Minamoto A, Nakashima E, Kumagami T, Kitaoka K, Kanamoto T, Kiuchi Y, Chang P, Fujii N, Shore RE: Meeting report: Radiation cataractogenesis: Epidemiology and biology. Radiation Research. (related to *Adult Health Study*)

⌘ Hsu WL, Tatsukawa Y, Neriishi K, Yamada M, Cologne JB, Fujiwara S: Longitudinal trends of total white blood cell and differential white blood cell counts of atomic bomb survivors. Journal of Radiation Research. (related to *Life Span Study*)

⌘ Shore RE, Neriishi K, Nakashima E: Epidemiologic studies of cataract risk at low-to-moderate radiation doses: (not) seeing is believing. Radiation Research.

⌘ Tsuneto A, Hida A, Sera N, Imaizumi M, Ichimaru S, Nakashima E, Seto S, Maemura K, Akahoshi M: Fatty liver incidence and predictive variables. Hypertension Research.

⌘ Yamada M: Follicle stimulating hormone and estradiol levels during perimenopause in a cohort of Japanese women: The Radiation Effects Research Foundation Adult Health Study. Menopause. New York. Nova Science Publishers.

⌘ Yamada M: TSH levels during the perimenopause: The Radiation Effects Research Foundation Adult Health Study. Menopause. New York. Nova Science Publishers.

Special Clinical Studies Oral Presentations

❖ Tatsukawa Y, Masunari N, Ohishi W, Yamada M, Yamane K, Fujiwara S. Relationship between metabolic syndrome and hyperhomocysteinemia. The 52nd Annual Meeting of the Japan Diabetes Society, 21–24 May 2009, Osaka

❖ Ohishi W, Hayashi T, Kusunoki Y, Nakashima E, Fujiwara S, Chayama K. Influence of *HLA-DRB1* Allele on the clearance and persistence of hepatitis C virus. The 45th Annual Meeting of the Japan Society of Hepatology, 4–5 June 2009, Kobe (related to *Immunology Studies*)

❖ Kiuchi Y. The projects in Hiroshima to elucidate the mechanism of glaucomatous optic neuropathy. The 91st Osaka University Alumni Reunion Meeting, 14 June 2009, Osaka

❖ Yamada M, Kasagi F, Mimori Y, Miyachi T, Ohshita T, Sasaki H. Smoking effects on mortality and dementia—Radiation Effects Research Foundation Adult Health Study. International Conference on Alzheimer's Disease, 11–16 July 2009, Vienna, Austria

❖ Ishida N, Ichimaru S, Hida A, Soda M, Akahoshi M, Nakamura T. Transportability of a regression model. The 3rd International Conference on Cancer Risk Assessment, 16–18 July 2009, Porto Heli, Greece

❖ Fujiwara S. Prediction of bone fracture risk among the Japanese by various parameters. The 27th Annual Meeting of the Japanese Society for Bone and Mineral Research, Japan-Korea Joint Symposium, 23–25 July 2009, Osaka

❖ Hsu WL, Neriishi K, Kakuma T, Araki Y. Application of joint modeling for growth model and time-to-event analysis. 2009 Joint Statistical Meetings, 1–6 August 2009, Washington DC, USA

❖ Fujiwara S, Masunari N, Chen P. Vertebral fracture status and the World Health Organization (WHO) risk factors for predicting osteoporotic fracture risk in Japan. The 31st Annual Meeting of the American Society of Bone and Mineral Research, 11–15 September 2009, Denver, Colorado, USA

❖ Neriishi K, Yokoyama T, Takamatsu M, Kiuchi Y, Tsuiki E, Uematsu M, Kumagami T, Kitaoka T, Minamoto A, Nakashima E, Hida A, Fujiwara S, Akahoshi M. Glaucoma study in atomic-bomb survivors. The 55th Annual Meeting of the Radiation Research Society, 4–7 October 2009, Savannah, Georgia, USA

❖ Kiuchi Y, Takamatsu M, Yokoyama T, Kumagami T, Uematsu M, Tsuiki E, Kitaoka T, Neriishi K, Nakashima E, Hida A, Fujiwara S, Akahoshi M. Glaucoma study in atomic-bomb survivors. The 63rd Annual Congress of the Japan Clinical Ophthalmology, 9–12 October 2009, Fukuoka

❖ Yamada M, Kasagi F, Mimori Y, Miyachi T, Ohshita T, Sasaki H. Reaction time as a predictor of mortality and dementia: Radiation Effects Research Foundation Adult Health Study. The 3rd Congress of the Asia Society Against Dementia, 12–13 October 2009, Seoul, Sourth Korea

❖ Fujiwara S. Symposium 6. Application of FRAX to the Japanese. 1. Prediction of fracture risks among the Japanese (general population). The 11th Annual Meeting of the Japan Osteoporosis Society, 14–16 October 2009, Nagoya

❖ Fujiwara S, Masunari N, Fukunaga M. Quantitative Ultrasound (QUS) measurements predicted for bone fracture independently of fracture risk assessment tool (FRAX). The 11th Annual Meeting of the Japan Osteoporosis Society, 14–16 October 2009, Nagoya

❖ Oyama H, Fujiwara S, Masunari N, Yamane K, Fukunaga M. Structural trends in proximal femur using Hip Structural Analysis and association between them and fragility hip fracture; Hiroshima cohort study. The 11th Annual Meeting of the Japan Osteoporosis Society, 14–16 October 2009, Nagoya

❖ Ohishi W, Tatsukawa Y, Fujiwara S, Masunari N, Yamada M, Tsuge M, Chayama K. Relationship between metabolic syndrome and liver dysfunction and/or fatty liver. The 13th Annual Meeting of the Japan Society of Hepatology, 14–17 October 2009, Kyoto (related to *Adult Health Study*)

❖ Ishida N, Ichimaru S, Nakamura T, Akahoshi M. On transportability of parameters and estimation of risks associated with metabolic syndrome. International Conference in Modeling Health Advances 2009, 20–22 October 2009, San Francisco, California, USA

❖ Ishida N, Ichimaru S, Nakamura T, Akahoshi M. Risk assessment using transported estimates correcting for the Berkson Type measurement errors. 2009 Non-Clinical Biostatistics Conference, 21–23 October 2009, Boston, Massachusetts, USA

❖ Yoshida K, Ohishi W, Chayama K, Kusunoki Y, Hayashi T. Effects of *NKG2D* polymorphism on hepatitis-C virus infection. The 39th Annual Meeting of the Japanese Society for Immunology, 2–4 December 2009, Osaka (related to *Immunology Studies*)

❖ Fujiwara S, Masunari N, Ohishi W. Relationship between serum undercarboxylated osteocalcin (ucOC) level and bone mineral density and bone loss. The 13th Meeting of Vitamin K & Aging, 20 February 2010, Tokyo

❖ Imaizumi M, Sera N, Ueki I, Horie I, Ando T, Usa T, Tominaga T, Ashizawa K, Maeda R, Nagataki S, Eguchi K. Findings of thyroid ultrasound and natural course of thyroid function in patients with subclinical hypothroidism. The 83rd Annual Meeting of the Japan Endocrine Society, 25–28 March 2010, Kyoto

❖ Tatsukawa Y, Masunari N, Yamada M, Hakoda M, Yamane K, Fujiwara S. Serum uric acid level and prevalence of metabolic syndrome: Adult Health Study in Hiroshima. The 14th International Congress of Endocrinology, 26–30 March 2010, Kyoto

Research Protocols 5-89 (Platform Protocol), A2-08

Histopathology Study

RP 5-89 Pathology studies in Hiroshima and Nagasaki. Revised research plan

Tokuoka S (RC), Yonehara S, Fujihara M, Ozasa K (EH), Soda M (EN), Suyama A (EN), Kodama K (CS)

The pathology program started at the inception of ABCC has undergone several major revisions through the years. Autopsy rates peaked at 40–45% in the early 1960s but subsequently declined and the autopsy program was terminated in 1988. In 1987, the pathology program was redesigned to conduct surgical pathoepidemiological follow-up studies of atomic-bomb survivors in order to verify diagnoses and to detect specific histologic, cytologic, or other tissue changes directly or indirectly attributable to irradiation (RP 9-88).

A large number of LSS autopsy (about 7,500 cases) and surgical tissue samples (about 13,000 cases) have been collected through the ABCC-RERF pathology program over the years. These archived tissues have been useful for molecular studies as well as usual pathological investigation. Many of the recent tissue samples are stored in the outside hospitals at which subjects in the LSS were diagnosed but are obtained under agreements signed between the hospitals and RERF. To satisfy the recent amendments of governmental ethical guidelines, an addendum to the RP is in preparation.

A new project for storage of histopathological specimens (tissue blocks) is planned in Hiroshima University and hospitals in Hiroshima areas, Nagasaki University and hospitals in Nagasaki areas, and RERF. It is chaired by Dr. Okubo and funded by the Ministry of Health, Labour and Welfare.

RP-A2-08 Histopathological identification of multiple primary cancers occurring in Nagasaki atomic-bomb survivors

Nakashima M, Soda M (EN), Suyama A (EN), Kasagi F (EH), Furukawa K (S), Sekine I, Yamashita S, Shibata Y, Kodama K (CS)

The development of multiple primary cancers (MPC) may be related to both exposure to carcinogenic factors and high cancer susceptibility. Thus, the relationship between A-bomb irradiation and the development of MPC among A-bomb survivors is an important issue in elucidating the influence of the bombings on carcinogenesis as a late health effect. In identifying MPC cases, it is essential to determine whether the second cancer is primary or metastatic.

The purpose of this protocol is to identify more accurately true MPC diagnoses vs. metastatic disease by modern histopathological methods as an initial step for evaluating the relationship between MPC and radiation exposure in A-bomb survivors. We will identify MPC cases histopathologically with immunohistochemical methods to differentiate primary and metastatic cases, using as subjects A-bomb survivors in Nagasaki in the Life Span Study (LSS) population. This study will contribute to accurate identification of cases in order to obtain further information that can be used for examining the effects of radiation exposure on cancer risks.

The RP was approved in May 2008. There were 648 patients who had two or more cancers which had been histologically diagnosed in a total of 6,305 primary-cancer patients bearing cancer among 38,107 in the Nagasaki LSS cohort between 1958 and 2003. After reviewing HE-stained tissue specimens and immunohistochemistry findings, we found 595 cases were MPC, 41 cases (7%) were not MPC cases, 10 cases were undetermined and 2 cases had not yet been reviewed among the 648 cases.

Research Protocol 5-02
Cell Biology Study

RP 5-02 Papillary thyroid carcinomas in residents of Hiroshima and Nagasaki who were exposed to A-bomb radiation as children: A study of *RET* gene rearrangements and other DNA changes potentially responsible for the origins and/or development of these tumors

Hamatani K (R), Eguchi H, Taga M (R), Ito R (R), Imai K (R), Nishi N (EH), Soda M (EN), Katayama H (IT), Cologne JB (S), Arihiro K, Hayashi Y, Nakachi K

Thyroid cancer is one of the malignancies most closely associated with ionizing radiation in humans. We have previously found that rearrangement of *RET* proto-oncogene could be induced in human thyroid cells by *in vitro* (10, 50, or 100 Gy) and *in vivo* X-ray irradiation (50 Gy). *RET* rearrangements occurred in 60–80% of thyroid cancers in post-Chernobyl children and also in patients with histories of radiation therapy. On the other hand, $BRAF^{V600E}$ gene mutation is also a well-known early event in adult-onset thyroid carcinogenesis. We hypothesize that the high incidence of papillary thyroid cancer (PTC) in adult survivors who were exposed to A-bomb radiation at young ages is in part a result of *RET* rearrangements and other chromosomal rearrangements induced by ionizing radiation. To examine that hypothesis, we are analyzing *RET/PTC* rearrangements, $BRAF^{V600E}$ mutation, and other alterations in cancer tissues of adult-onset PTC patients from the LSS cohort.

We examined point mutations of *BRAF, K-RAS*, *N-RAS,* and *H-RAS* genes as well as rearrangements of *RET*, *NTRK1,* and *BRAF* genes, in 105 adult-onset PTC patients (including 26 patients not exposed to radiation) from A-bomb survivors. The frequency of PTC cases with chromosomal rearrangements, mainly *RET/PTC* rearrangements, significantly increased with radiation dose, while point mutations including $BRAF^{V600E}$ significantly decreased with radiation dose. The relative frequency of PTC cases with non-detected gene alterations (i.e., no alterations in the *RET*, *NTRK1*, *BRAF*, and *RAS* genes) increased with radiation dose and numbered 25, in which we recently found a new type of rearrangement, i.e., rearranged anaplastic lymphoma kinase

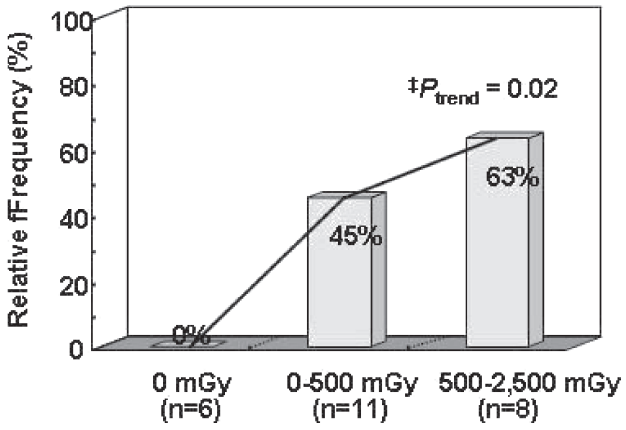


Figure. Increased frequency of rearranged *ALK* in PTC cases with non-detected alterations with radiation dose. "n" indicates the number of PTC case with non-detected gene alterations. ‡ Cochran-Armitage test.

(*ALK*) gene for the first time in PTC, although identification of the partner genes is needed. Rearrangement of *ALK* was found in 10 of the 19 exposed PTC cases with non-detected gene alterations, but none in 6 non-exposed PTC cases (Figure).

Cell Biology Study Publications

RERF Report (RR)

♦ Hamatani K, Eguchi H, Mukai M, Koyama K, Taga M, Ito R, Hayashi Y, Nakachi K: Improved method for analysis of RNA present in long-term preserved thyroid cancer tissue of atomic bomb survivors. Thyroid 2010 (January); 20(1):43–9.

 (RR 7-08)

[Abstract] ***Background.*** Since many thyroid cancer tissue samples from atomic bomb (A-bomb) survivors have been preserved for several decades as unbuffered formalin-fixed, paraffin-embedded specimens, molecular oncological analysis of such archival specimens is indispensable for clarifying the mechanisms of thyroid carcinogenesis in A-bomb survivors. Although *RET* gene rearrangements are the most important targets, it is a difficult task to examine all of the 13 known types of *RET* gene rearrangements with the use of the limited quantity of RNA that has been extracted from invaluable paraffin-embedded tissue specimens of A-bomb survivors. In this study, we established an improved 5′ rapid amplification of cDNA ends (RACE) method using a small amount of RNA extracted from archival thyroid cancer tissue specimens. ***Methods.*** Three archival thyroid cancer tissue specimens from three different patients were used as in-house controls to determine the conditions for an improved switching mechanism at 5′ end of RNA transcript (SMART™) RACE method; one tissue specimen with *RET/PTC1* rearrangement and one with *RET/PTC3* rearrangement were used as positive samples. One other specimen, used as a negative sample revealed no detectable expression of the *RET* gene tyrosine kinase domain. ***Results.*** We established a 5′ RACE method using an amount of RNA as small as 10 ng extracted from long-term preserved, unbuffered formalin-fixed, paraffin-embedded thyroid cancer tissue by application of SMART technology. This improved SMART RACE method not only identified common *RET* gene rearrangements, but also isolated a clone containing a 93-bp insert of rare *RET/PTC8* in RNA extracted from formalin-fixed, paraffin-embedded thyroid cancer specimens from one A-bomb survivor who had been exposed to a high radiation dose. In addition, in the papillary thyroid cancer of another high-dose A-bomb survivor, this method detected one novel type of *RET* gene rearrangement whose partner gene is acyl coenzyme A binding domain 5, located on chromosome 10p. ***Conclusion.*** We conclude that our improved SMART RACE method is expected to prove useful in molecular analyses using archival formalin-fixed, paraffin-embedded tissue samples of limited quantity.

Other Journal Publications

♦ Hamatani K, Takahashi K, Mukai M: Mechanisms of development of human adult-onset radiation-associated papillary thyroid cancer—Characteristics in early events. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2009 (December); 44(4):379–95. (Japanese)

♦ Hirai Y: Effective screening of heterozygotes for ATM mutations. Hiyama E, Hiyama K, eds. Clinical Application of Molecular Diagnosis—Cancer, Radiation Effects, and Human Diseases. Kerala, India: Transworld Research Network; 2009, pp 53–63.

♦ Hirai Y: How to obtain enough DNA from a limited amount of clinical materials. Hiyama E, Hiyama K, eds. Clinical Application of Molecular Diagnosis—Cancer, Radiation Effects, and Human Diseases. Kerala, India: Transworld Research Network; 2009, pp 9–20.

Cell Biology Study Oral Presentations

❖ Hamatani K, Takahashi K, Taga M, Ito R, Hayashi Y, Nakachi K. Molecular oncology study on adult-onset papillary thyroid cancer among atomic-bomb survivors. The 34th Annual Meeting of the European Thyroid Association, 5–9 September 2009, Lisbon, Portugal

❖ Hamatani K, Takahashi K, Taga M, Ito R, Nakachi K. Molecular oncological analysis of adult-onset papillary thyroid cancer among atomic-bomb survivors. The 68th Annual Meeting of the Japanese Cancer Association, 1–3 October 2009, Yokohama

❖ Takahashi K, Taga M, Ito R, Nakachi K, Hamatani K. Chromosomal rearrangements and amplification of *PIK3CA* gene in papillary thyroid cancer among atomic-bomb survivors. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Ito R, Eguchi H, Hamatani K, Taga M, Takahashi K, Oue N, Yasui W, Nakachi K. Genetic alterations relative to microsatellite instability in colorectal cancer among atomic-bomb survivors. The 20th Annual Meeting of the Japanese Society for Gastroenterological Carcinogenesis, 26–27 November 2009, Hiroshima

Research Protocols 1-10, 2-07, 1-07, 1-97 and 2-01, 5-85 and 1-01

Biochemical Genetics Studies

RP 1-10 Estimation of genetic effects of radiation in male germ cells of mice: Study for assessment of high-density microarray CGH platform

Asakawa J (G), Kodaira M (G), Cullings HM (S), Shimada Y, Nakamura N (CS)

Planning a full-scale genetic study in the offspring of A-bomb survivors is a difficult task because, compared with animal studies that used large radiation doses, A-bomb survivors received much smaller doses on the average, and radiation sensitivity of human germ cells is not well understood. On the other hand, animal data are not free from problems either; they are mostly limited to Russell's 7 loci and practically no information exists on the whole genome.

Recently, high-density (HD) microarray slides have become commercially available, which enables us to conduct high-resolution comparative genomic hybridization (CGH) studies. This HD-array allows us to examine copy number changes (deletions and/or amplifications) at every 1 kb throughout the whole genomes between two DNA samples from the same species (examination of a total of 2.1 million DNA loci).

In the proposed study, we will first conduct a model experiment to estimate the detection rate and the size of the smallest copy number variation (CNV) by examining the polymerase chain reaction (PCR)-confirmed CNVs existing in two different mouse genomes. Following the characterization of the methods, we will undertake a small-scale screening study of 30 DNA samples from F_1 mice derived from irradiated spermatogonial cells and 30 samples from control F_1 mice to obtain crude information on the spontaneous as well as radiation-induced mutation rates that are essential for planning a larger scale study for determining the dose response. Finally, it is crucially important to know if radiation exposure may induce not only deletions but also amplifications in this animal model study.

RP 2-07 Study on genetic effects of radiation by array comparative genome hybridization (array-CGH) method—validation of ability of the methodology for the genetic study

Takahashi N (G), Satoh Y (G), Kodaira M (G), Katayama H (IT), Kodama Y (G), Cologne JB (S)

For the large population of F_1 offspring of A-bomb survivors, the bacterial artificial chromosome DNA-microarray-based comparative genome hybridization (BAC-aCGH) method was selected and refined in order to effectively identify "radiation-induced *de novo* mutations." The results of a pilot study revealed that our BAC-aCGH system could detect changes in copy number variation (CNV) with good sensitivity (Takahashi et al., *Annals of Human Genetics* 2008; 72:193–204 and Takahashi et al., *Cytogenetic Genome Research* 2008; 123:224–33).

In this RP, the genomic DNA from 225 offspring born to parents, at least one of whom was exposed to high doses (≥1.0 Gy) of radiation, had been screened using our BAC-aCGH system that consists of approximately 2,500 BAC clones. We finished a population survey, and found 93 rare CNVs (with frequency <1%). Family studies conducted for rare CNVs demonstrated that three CNVs were found to be "putative *de novo* mutants" because they were not observed in either parent. In order to determine the parental origin of the *de novo* mutations, we examined DNA from father-mother-child trios using the Affymetrix SNP Array 6.0.

The results revealed that all three mutations originated in the gametes from highly exposed fathers (about 2 Gy). The statistical analysis showed that "there is evidence of a statistically significant effect of radiation exposure on mutation frequency." However, this number of mutations (three) is too small to reach a firm conclusion as to whether the mutation rate of the exposed group is significantly higher than that in the unexposed group. Therefore, this study will be superseded by a new RP in which the study will continue using the high-density array system to increase the number of loci examined. The RP is under institutional review.

RP 1-07 Estimation of genetic risk of radiation on immature oocytes of rats by using two-dimensional DNA analysis: An animal model for human female exposure

Asakawa J (G), Kamiguchi Y, Nakamura N (CS), Katayama H (IT), Cullings HM (S)

Up till now, there has been no suitable animal model to study the genetic effects of female gonadal exposure. Mouse immature oocytes are highly sensitive to ionizing radiation and readily die by apoptosis at rather modest doses. As we recognized the powerful nature of the 2-DE method for screening mutations in any mammals whose genomic information is unknown, we searched for the most appropriate laboratory animal model of radiation mutagenesis in human female gametes. We considered several key requirements: (a) the intrinsic radiosensitivity of immature oocytes, (b) availability of several inbred strains bearing variation in base sequences so that the parental origin of mutations in the F_1 can be assessed, and (c) ease in raising pups. After evaluating the suitability of various rodent species, we reached a tentative conclusion that a rat model may be the best animal model currently available, especially since rat immature oocytes are less sensitive to ionizing radiation than mouse oocytes.

Dr. Kamiguchi (Asahikawa Medical College) prepared F_1 rats after mating 2.5 Gy-exposed females (strain SD) with unirradiated males (strain BN) for our future screening of mutations using the 2-DE method. We prepared two types of gels, 1–5-kb fragments and 5–10-kb fragments, per animal. We analyzed a total of 3,000 gels prepared from 1,500 F_1 rats. In other word, we analyzed about 2.2 million spots (loci), each derived from maternal SD or paternal BN strains. A total of 24 mutations, 13 from the control and 11 from the 2.5-Gy exposed group, were confirmed as newly arisen germline mutations by the results on DNA from three different tissues (spleen, kidney and liver) and by family studies. We also cloned and sequenced 21 normal DNA fragments where mutations were detected (three DNA fragments are not yet cloned). Four mutations, two each from the control and exposed groups, were deletions, but none of them occurred in the exposed maternal alleles. The

majority of the mutations (11/13 mutations in the control and 9/11 in the exposed group) seemed to have occurred at microsatellite sequences (i.e., alterations in the number of repeats and probably spontaneous in origin). In summary, we have observed no indication for the transgenerational effects of radiation following exposure of immature oocytes of rats to 2.5 Gy of γ rays.

RP 1-97 Pilot study of the genetic background of the Adult Health Study (AHS) population: Identification of markers in potential candidate genes associated with hypertension

RP 2-01 The acquisition of signed informed consent forms from the donors (or their proxies) for genomic studies conducted either at RERF or at other research institutes as collaborative study using previously collected blood samples (Addendum to RP 1-97)

Takahashi N (G), Murakami H (G), Yamada M (CH), Kasagi F (EH), Kodama K (CS)

The aim of this study is to test the hypothesis that differences in genetic background in the survivors modify the radiation risk for non-cancer diseases. For this purpose, we established a small-scale case-control study in the AHS and initially sought to identify key polygenic elements that may be predisposing to hypertension. A nationwide project started in 2000 served to complement our effort by initiating a large-scale search for hypertension-related genes. From that effort, it was found that 38 polymorphisms of single nucleotide polymorphisms (SNPs) were significantly associated with hypertension (about 2,300 study subjects consisting of 1,100 cases and 1,200 controls). A summary paper was published (Kohara et al., *Hypertension Research* 2008; 31:203–12).

The consortium has been continuing the confirmation studies for the SNPs which were significantly associated with hypertension in the first screening. For this purpose, a larger population consisting of about 14,000 individuals (7,000 cases and 7,000 controls) was used. One SNP was significantly associated with hypertension. Odds ratio is 1.31 ($p = 1 \times 10^{-11}$). This SNP was also confirmed by another population consisting of about 34,000 Caucasoid. Moreover, the result was also confirmed by a knock-out mouse study. After the accumulation of the data, the SNP information will be applied to the AHS population for the study of radiation-associated cardiovascular diseases of A-bomb survivors.

RP 5-85 Culture of permanent lymphocyte cell lines as sources of biological samples for investigation of genetic effects of radiation on children of atomic-bomb survivors

RP 1-01 The acquisition of signed informed consent forms from the donors (or their proxies) for whom permanent cell lines have been established (Addendum to RP 5-85)

Takahashi N (G), Satoh Y (G), Murakami H (G), Katayama H (IT), Fujiwara S (CH), Akahoshi M (CN)

The purpose of this RP is to establish lymphoblastoid cell lines by transformation of B-cells from members (parents and children) of about 1,300 families as biological resources for current and future genetic studies, e.g., studies of mini- and microsatellite markers and DNA microarrays that can be used to address the issue of transgenerational radiation effects. In addition, collection of blood samples and establishment of permanent cell lines from those individuals omitted from the original cohort, but later added, are also being done.

No written informed consent was obtained when this RP was originally approved in 1985. In order to avoid possible ethical issues concerning work in the future, signed consent forms are now being collected from all participants (RP 1-01).

We are continuing the effort to collect blood samples from as many family members as possible. Although we have been successful in obtaining informed consent from the majority of blood donors, we need to make a continuing effort to obtain written consent from additional donors. The cumulative numbers of children from whom cell lines were established and from whom signed informed consent forms were obtained are shown in the Table.

Table. The cumulative numbers of children with established cell lines and signed informed consent forms

Father's dose (Gy)	Mother's dose (Gy)				
	≥1	0< <1	0	Unknown	Total
≥1	2	24	146	2	174
0< <1	5	63	199	9	276
0	109	280	608	46	1,043
Unknown	0	7	30	0	37
Total	116	374	983	57	1,530

We are making efforts to safely maintain our archival specimens. Moreover, whenever the stocks of the frozen EB-transformed samples are utilized for any study, we replenish them and return them to the archive.

Biochemical Genetics Studies Publications

Journal Publications

♦ Asakawa J, Kodaira M, Katayama H, Cullings HM, Nakamura N: A genetic risk estimate of radiation in mice based on whole genome scanning by two-dimensional DNA gel. Skopek J, ed. 37th Annual Meeting of the European Radiation Research Society. Bologna: Medimond s.r.l.; 2009, pp 41-3. (Proceedings of the 37th Annual Meeting of the European Radiation Research Society, Prague, Czech Republic, 26–29 August 2009)

♦ Asakawa J, Nakamura N, Shiroishi T: Detection of hypermutable AT-rich sequences among mice; Fingerprints of micro-evolution. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2009 (December); 44(4):407–18. (Japanese)

♦ Kodaira M: The use of human minisatellite markers for the evaluation of genetic risk. Hiyama E, Hiyama K, eds. Clinical Application of Molecular Diagnosis—Cancer, Radiation Effects, and Human Diseases. Kerala, India: Transworld Research Network; 2009, pp 35–51.

♦ Takahashi N: Phosphoglucomutase (PGM). Nippon Rinsho [Japanese Journal of Clinical Medicine] 2010 (January); 68(Suppl 1):864–7. (Japanese)

♦ Takahashi N, Satoh Y, Kodaira M, Katayama H: Large-scale copy number variants (CNVs) detected in different ethnic human populations. Kehrer-Sawatzki H, Cooper DN, eds. Copy Number Variation and Disease. Basel: Karger; 2009, pp 224–33.

Biochemical Genetics Studies Oral Presentations

❖ Asakawa J, Kamiguchi Y, Katayama H, Cullings HM, Nakamura N. Estimation of genetic risk of radiation on female rats. The 34th Annual Meeting of the Chugoku Area Radiation Research Society, 29 July 2009, Hiroshima

❖ Asakawa J, Kodaira M, Katayama H, Cullings HM, Nakamura N. A genetic risk estimate of radiation in mice based on whole genome scanning by two-dimensional DNA gel. The 37th Annual Meeting of the European Radiation Research Society, 26–29 August 2009, Prague, Czech

❖ Satoh Y, Sasaki K, Fukuba I, Hiyama E, Imanaka M, Shimoichi Y, Kaneko J, Kodaira M, Takahashi N. Determination of parental origin by SNP array of *de novo* copy number mutations identified in offspring of atomic-bomb survivors. The 59th Annual Meeting of the American Society of Human Genetics, 20–24 October 2009, Honolulu, Hawaii, USA

❖ Takahashi N, Satoh Y, Kodaira M, Sasaki K, Kodama Y, Shimoichi Y, Kaneko J, Miura A, Imanaka M, Hiyama E, Fukuba I, Katayama H, Cologne JB. Study of genetic effects of atomic-bomb radiation using BAC-aCGH: *De novo* copy number mutants detected by aCGH in a Japanese population. The 59th Annual Meeting of the American Society of Human Genetics, 20–24 October 2009, Honolulu, Hawaii, USA

❖ Asakawa J, Kodaira M, Katayama H, Cullings HM, Nakamura N. A genetic risk estimate of radiation in mice by two-dimensional DNA electrophoresis. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Kodaira M, Asakawa J. Analysis of mice deletion mutations by a CGH approach with high-density microarray (II). The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Satoh Y, Sasaki K, Fukuba I, Hiyama E, Imanaka M, Shimoichi Y, Kaneko J, Kodaira M, Takahashi N. *De novo* copy number mutation occurred on the chromosome derived from the father. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Takahashi N, Satoh Y, Kodaira M, Sasaki K, Kodama Y, Shimoichi Y, Kaneko J, Miura A, Imanaka M, Hiyama E, Fukuba I, Katayama H, Cologne JB. Study of trans-generational effects of atomic-bomb radiation: Copy number variants (CNVs) were used as markers. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Takahashi N, Satoh Y, Kodaira M, Kodama Y, Sasaki K, Shimoichi Y, Kaneko J, Miura A, Imanaka M, Hiyama E, Fukuba I, Katayama H, Cologne JB. Study of trans-generational effects of atomic-bomb radiation by a bacterial artificial chromosome (BAC) DNA micro-array based comparative genomic hybridization (BAC-aCGH). The 32nd Annual Meeting of the Molecular Biology Society of Japan, 9–12 December 2009, Yokohama

Research Protocols 6-09, 1-08, 6-00, 8-93, A4-09, A2-09

Cytogenetics Studies

RP6-09 Evaluation of the nonmelanoma skin cancer risk among heterozygotes bearing a founder mutation allele unique to a Japanese population at *xeroderma pigmentosum group A (XPA)* gene

Hirai Y (G), Nakamura N (CS), Noda A (G), Cullings HM (S), Ozasa K (EH), Tokuoka S, Yonehara S, Fujihara M, Moriwaki S, Nishigori C, Mabuchi K, Kraemer KH, Land CE, Kodama Y (G)

The frequency of patients with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is usually low, but carriers (heterozygotes) are not rare. However, there is little data regarding cancer risk in the carriers of the heterozygotes, as they are generally difficult to identify. This study will focus on a founder mutation allele of *XPA* gene, which is an inactive mutation allele known to cause severe disease phenotypes under homozygous conditions. The mutation heterozygotes were found in about 1% of the general population (9/1,020 in our previous study; Hirai et al, *Mutation Research* 2006; 41:231–7), which is unique to Japanese. The condition provides a unique advantage in effective screening of such carriers. The purpose of the study is to elucidate the frequency of carriers of an inactive, founder mutation allele of the *XPA* gene among nonmelanoma skin cancer patients, and compare the frequency to the frequency in a general population. We plan to examine ~1,000 skin cancer cases for this purpose. This RP was approved on 22 December 2009. The study has just begun.

RP 1-08 Establishment of a recombinant mouse model for assessment of genetic effects of radiation at low doses

Noda A (G), Hirai Y (G), Kodama Y (G), Cullings HM (S), Nakamura N (CS)

The purpose of this research proposal is to create useful experimental animal systems by applying modern gene manipulation techniques for obtaining supplementary information that is difficult to collect in humans or ordinary experimental animals; e.g., measurement of genetic effects of radiation at somatic and germ cell levels *in vivo*. The system consists of the idea that the living mutant cells arising in tissues will produce green fluorescence proteins (GFP), which allows us not only to detect the cell types affected but also to measure the mutation frequencies at different dose levels to derive dose responses. We plan to manipulate a specific gene locus, the HPRT gene as a model; either by creating a tandem duplication of the endogenous HPRT sequences containing a GFP gene in frame at the 3′ end, or by inserting a gene into the HPRT locus whose products suppress expression of the GFP gene that was inserted somewhere in the genome. In the former case, loss of one copy of the duplication (reversion mutation) will let the HPRT gene recover the wild type function, thus allowing expression of the hybrid HPRT-GFP protein (the cells become green fluorescence positive). In the latter case, inactivating mutations of the suppressor gene inserted

within the HPRT locus make the cells fluorescent. Thus far, we have succeeded in creating knock-in mice carrying a partial duplication of the HPRT gene sequence flanked with the GFP gene ($HPRT^{dup}$-GFP). In various tissue sections, e.g., pancreas and testis, spontaneously arising mutants are detected *in situ*. We are now verifying the usefulness of the mice for analyzing genetic effects of radiation exposure.

RP 6-00 Molecular alterations in early-onset breast and ovarian cancers among atomic bomb survivors

Hirai Y (G), Tokuoka S, Cologne JB (S), Mabuchi K, Land CE, Noda A (G), Kodama Y (G), Nakamura N (CS)

The purpose of the study is to test the hypothesis that the high incidence of early-onset breast cancers among A-bomb survivors may be due to the damaging effect of A-bomb radiation on the normal allele of a breast cancer susceptibility gene in women who are heterozygous carriers of germline mutations of the gene. So far, frequent founder mutations among Japanese did not explain the observed high risk. The study is currently inactive and we plan to re-evaluate the research strategies. A part of this study will be incorporated into a pathological study of breast cancers which plans to determine intrinsic subtypes (Addendum to RP 5-08, Yonehara et al, which is currently under review).

RP 8-93 Cytogenetic study in the Adult Health Study population by fluorescence *in situ* hybridization (FISH)

Kodama Y (G), Hamasaki K (G), Noda A (G), Kodaira M (G), Takahashi N (G), Kusunoki Y (R), Shimizu Y (EH), Nakashima E (S), Cullings HM (S), Misumi M (S), Nakamura N (CS)

FISH examination in AHS population

Preliminary data from 1,441 survivors (900 from Hiroshima and 541 from Nagasaki) are summarized as follows: (a) a wide scatter of individual translocation frequencies was observed when plotted against DS02 doses as seen in the previous Giemsa staining study; (b) the city difference became much smaller now using FISH, and the difference is only marginally significant (Figure); (c) factory workers in Nagasaki and survivors who were exposed outdoors but were shielded by houses in both cities had significantly lower dose responses than people who were exposed in Japanese houses; (d) the reduced intercity difference suggests that the previous city difference by the Giemsa method was mainly due to different aberration detection rates between Hiroshima and Nagasaki laboratories; (e) survivors exposed at 0–5 years of age had a shallower dose-response slope compared to that of adults.

Lack of cytogenetic dose response for *in utero* population

We have previously found that survivors who were exposed *in utero* showed almost no dose response for translocation frequency when examined at age 40, which was confirmed by subsequent mouse experiments. In order to see whether the above finding is unique to hematopoietic cells, we examined chromosome aberration frequencies in rat mammary epithelial cells following fetal irradiation. The results indicated that irradiated fetuses recorded radiation damage like their mothers when examined at 5–8 weeks of the age. Thus, it is quite clear that the lack of translocation dose response following fetal exposure is tissue-dependent.

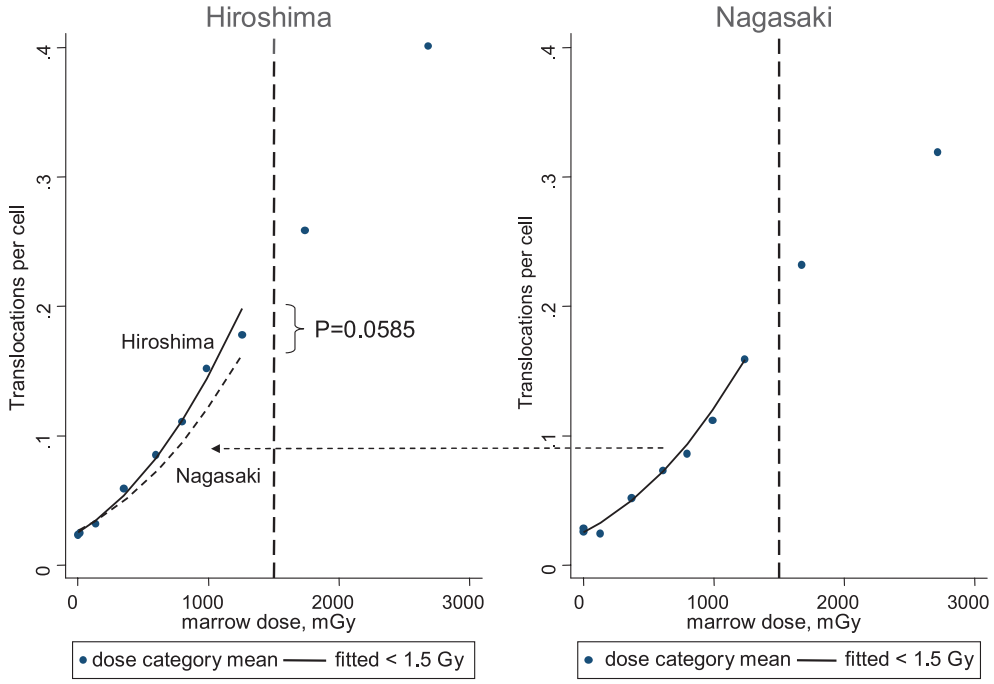


Figure. Comparison of dose-response relationships between Hiroshima and Nagasaki. Overall slope of the dose response in Nagasaki was 83% of that in Hiroshima, which is much more similar than was previously seen with the Giemsa staining. Statistical analysis indicates that the city difference is now only marginally significant (p = 0.0585).

Study of cytogenetic instability *in vitro*

Our previous study on clonal lymphocyte populations in A-bomb survivors did not indicate the presence of genetic instability *in vivo*. In a follow-up study, we propagated blood lymphocytes *in vitro* for detection of newly-induced additional translocations using multi-color FISH (mFISH). We examined nearly 7,000 metaphases by mFISH. The results did not show a statistically significant increase in the frequency of additional sporadic translocations among the clonally-expanded lymphocytes from the high-dose exposed survivors when compared with that of control subjects. The paper describing the results was published in 2009 (Hamasaki et al., *Radiation Research* 2009; 172:234–43). This is a collaborative study with the Immunology group in the Department of Radiobiology/Molecular Epidemiology.

RP-A4-09 Detection of unrepairable DNA damages (DNA double strand breaks) in the past-irradiated cells and tissues

Noda A (G), Hirai Y (G), Nakamura N (CS), Kodama Y (G)

In cultured normal human fibroblasts irradiated at higher doses, we have successfully detected persistent, unrepairable DNA-double strand breaks, as large γH2AX/ATM/53BP1 foci (ionizing radiation-induced foci; IRIF) in nuclei. Since the unrepairable IRIFs appear to persist as long as the culture continues, we are speculating that radiation-induced unrepairable damages are permanently retained in non-apoptotic and non-dividing quiescent cells in the irradiated individuals. This new type–A RP was launched this fiscal year for the biochemical characterization of the unrepairable IRIFs that are distinct from repairable ones so that we could re-investigate the actual doses received by archival tissues of A-bomb survivors.

RP-A2-09 Comprehensive analysis of radiation-induced genetic-damage regions in human peripheral blood T cells using comparative genomic hybridization (CGH) and cytogenetic techniques

Honma M, Ukai A, Hamasaki K (G), Kodama Y (G), Kusunoki Y (R)

This collaborative study was planned in response to the request by Dr. Homma, Japan National Institute of Health Sciences (NIHS). The request is based upon a background of information showing that ionizing radiation induces various types of structural alterations in the genome, but that little is known about how large chromosomal regions are altered and what types of genetic alterations preferentially remain in a normal cell following a given dose of ionizing irradiation. To comprehensively analyze damaged genome regions following radiation exposure, DNA from human peripheral blood T-cell populations clonally propagated after *in vitro* X-irradiation will be assessed by a comparative genomic hybridization (CGH) method with DNA from unirradiated blood mononuclear cells. Mutated genome regions that will be identified by this CGH assay will further be assessed for their relevance to chromosomal aberrations by G-banding and multi-color fluorescence *in situ* hybridization (mFISH) methods. Since each clonal population from irradiated blood of an individual will be analyzed in comparisons with unirradiated blood cells of the same individual, radiation-induced genome alterations can extensively be evaluated at a single-cell level. Because normal, untransformed cells will be analyzed, genome alterations observed in this study will mostly be specific to radiation exposure and may partly be involved in molecular events in the process of radiation carcinogenesis.

Peripheral blood mononuclear cells from one healthy volunteer were irradiated with 1 Gy of X-rays *in vitro*, and 27 T-cell colonies of single-cell origin were collected. Cells from those T-cell colonies were sent to NIHS. CGH analyses with the DNA extracted from the cells are under way.

Cytogenetics Studies Publications

RERF Report (RR)

♦ Hamasaki K, Kusunoki Y, Nakashima E, Takahashi N, Nakachi K, Nakamura N, Kodama Y: Clonally expanded T lymphocytes from atomic bomb survivors *in vitro* show no evidence of cytogenetic instability. Radiation Research 2009 (August); 172(2):234–43.

[Abstract] Genomic instability has been suggested as a mechanism by which exposure to ionizing radiation can lead to cancer in exposed humans. However, the data from human cells needed to support or refute this idea are limited. In our previous study on clonal lymphocyte populations carrying stable-type aberrations derived from A-bomb survivors, we found no increase in the frequency of sporadic additional aberrations among the clonal cell populations compared with the spontaneous frequency *in vivo*. That work has been extended by using multicolor FISH (mFISH) to quantify the various kinds of chromosome aberrations known to be indicative of genomic instability in cloned T lymphocytes after they were expanded in culture for 25 population doublings. The blood T cells used were obtained from each of two high-dose-exposed survivors (>1 Gy) and two control subjects, and a total of 66 clonal populations (36 from exposed and 30 from control individuals) were established. For each clone, 100 metaphases were examined. In the case of exposed lymphocytes, a total of 39 additional *de novo* stable, exchange-type aberrations [translocation (t) + derivative chromosome (der)] were found among 3,600 cells (1.1%); the corresponding value in the control group was 0.6% (17/3,000). Although the ratio (39/3,600) obtained from the exposed cases was greater than that of the controls (17/3,000), the difference was not statistically significant (P = 0.101). A similar lack of statistical difference was found for the total of all structural chromosome alterations including t, der, dicentrics, duplications, deletions and fragments (P = 0.142). Thus there was no clear evidence suggesting the presence of chromosome instabilities among the clonally expanded lymphocytes *in vitro* from A-bomb survivors.

Other Journal Publication

♦ Hamasaki K, Kusunoki Y, Nakashima E, Takahashi N, Nakachi K, Nakamura N, Kodama Y: Genomic instability in clonally expanded T-lymphocytes from A-bomb survivors in vitro. Hoshasen Seibutsu Kenkyu [Radiation Biology Research Communications] 2009 (December); 44(4):396–406. (Japanese) (related to *Immunology Studies*)

Cytogenetics Studies Oral Presentations

❖ Kodama Y, Nakano M, Ohtaki K, Cullings HM, Misumi M, Nakamura N. Translocation analysis in lymphocytes of A-bomb survivors: The city difference in dose response was much reduced with FISH. The 34th Annual Meeting of the Chugoku Area Radiation Research Society, 29 July 2009, Hiroshima

❖ Noda A, Oomine H, Hirai Y, Kodama Y, Nakamura N. Radiation dose and dose rate effects on double strand break (DSB) generation as revealed by H2AX foci measurements. The 34th Annual Meeting of the Chugoku Area Radiation Research Society, 29 July 2009, Hiroshima

❖ Kodama Y, Nakano M, Ohtaki K, Cullings HM, Misumi M, Nakamura N. Re-evaluation of translocation frequency in lymphocytes of A-bomb survivors by fluorescence *in situ* hybridization (FISH). The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Noda A, Oomine H, Hirai Y, Kodama Y, Nakamura N. Formation and structure of DSB-repair foci derived from unrepairable DSBs in irradiated normal human cells. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Nakamura N, Nakano M, Ohtaki K, Niwa O, Toyoshima M, Nakashima E, Shimada Y, Nishimura M, Yoshida MA, Nakata A, Kodama Y. Fate of chromosomally aberrant cells following fetal irradiation. Kids Workshop 2009 in National Institute of Radiological Sciences, 14–17 December 2009, Chiba

❖ Kodama Y. Biological dosimetry study in atomic-bomb survivors. Symposium on Urgent Medical Treatments for Radiological Accidents, 22 January 2010, Tokyo

❖ Kodama Y. Biodosimetric study of atomic-bomb survivors in Radiation Effects Research Foundation. International Conference on Cytogenetic Biodosimetry and Network in Hirosaki University, 19 March 2010, Aomori

Research Protocols 3-02, 4-75 (Platform Protocol)
F_1 Studies—Genetic Effects of Atomic Radiation on Children Born to Bombing Survivors

RP 3-02 Health effects study of the children of A-bomb survivors: Mail survey

Suyama A (EN), Furukawa K (S), Sakata R (EH), Grant EJ (EH), Kasagi F (EH), Ozasa K (EH), Kodama K (CS), Watanabe T (EH), Fujiwara S (CH), Cologne JB (S)

Studies of genetic effects have been a primary focus of the ABCC and RERF research program since its inception more than 50 years ago. Among the surviving members of the F_1 mortality cohort, a mail survey cohort was selected that included 24,673 members whose *koseki* and current address were in the catchment areas of the clinical Adult Health Study plus a small number of children of high-dose parents whose *koseki* was outside the catchment area and whose current address was in the catchment area. This survey was designed primarily to ascertain baseline epidemiological data on the F_1 subjects, and secondarily to identify F_1 cohort members willing to participate in clinical health examinations to investigate possible relationships of parental radiation doses to adult-onset noncancer diseases in the F_1 cohort.

The mail survey cohort consisted of 13,389 males and 11,284 females. The survey was carried out between 2000 and 2006. At the end of the survey, 16,756 (68%) subjects responded, 7,584 (31%) did not respond, and the rest were outside of the contact area, address unknown, or deceased. A total of 14,145 (57%) subjects agreed to participate in the health examination and 11,951 (71% of questionnaire respondents) actually came to RERF for a clinical examination. We made a final report brochure that summarized parts of the questionnaire and the F_1 clinical study and sent it to the survey respondents as a thank-you for their participation.

We published the results of the F_1 clinical study analysis, that included covariates from the mail survey data, investigating possible associations of parental radiation dose to F_1 multifactorial diseases (Fujiwara, Suyama, et al., *Radiation Research* 2008; 170:451–7). We will conduct the descriptive analysis of the full mail survey data and prepare a report for publication.

RP 4-75 Research plan for RERF studies of the potential genetic effects of atomic radiation; Hiroshima and Nagasaki. Part 1. Mortality study of children born to atomic bomb survivors

Suyama A (EN), Furukawa K (S), Sakata R (EH), Kasagi F (EH), Grant EJ (EH), Cullings HM (S), Shimizu Y (EH), Ozasa K (EH), Kodama K (CS), Cologne JB (S)

Since somatic and germinal mutations are thought to promote cancer and non-cancer disease development through numerous mechanisms, one might infer that possible radiation-induced, germinal mutations among A-bomb survivors would increase risk of cancer and non-cancer diseases in the F_1 generation. Although several experimental studies have found fairly large effects of radiation on mutation rates in the F_1 generation, others have reported such induced mutations are very rare. So an inquiry into the genetic effects of parental exposure in

humans on the mortality and cancer incidence of their children is an important and timely undertaking.

The F_1 mortality cohort target sample consisted of 76,814 subjects. They were selected from the children born from May 1946 through December 1984 to parents with a variety of A-bomb radiation exposures ranging from those known not to be in the city at the time of the bombing to those who were heavily exposed. About 41,000 F_1 subjects could be included in the mortality and cancer incidence parental dose-response analyses after appropriate exclusions due to missing parental dose information, etc.

The follow-up period of the current analyses was May 1946 to December 2003. During this period, 1,745 persons died and we have confirmed 418 cases of solid tumor, 57 cases of hematopoietic tumor, and 1,270 cases of noncancer diseases (infectious diseases 260, respiratory diseases 164, digestive diseases 230, circulatory diseases 285, and other diseases 331) in the F_1 cohort. Disease mortality rates were examined in relation to individual paternal and maternal gonadal doses using a Poisson regression model adjusted by city, age, birth-year, parental age at exposure, and parental age at child's birth. To date, neither non-cancer nor cancer mortality is significantly associated with paternal or maternal dose, but the F_1 cohort is still young (mean age <50) and has not yet expressed most of their eventual disease risk.

We will submit a draft paper of updated F_1 mortality and incidence followed-up to 2003 and will begin analyses based on cancer incidence follow-up to 2003.

Research Protocols 2-09, 1-09, 5-08, 4-07, 1-06, 2-04, 1-04, 6-02, 2-91 and 2-02, 3-94, 1-94, 2-92, 6-91, 9-88, 2-86, 29-60, A12-08, A5-08

Special Cancer Studies

RP 2-09 Study on secondary cancer risks after radiotherapy among A-bomb survivors

Yoshinaga S, Soda M (EN), Akahane K, Doi K, Moriwaki (EH), Hsu WL (S), Hida A (CN), Yamada M (CH), Katayama H (IT), Shimada Y, Fujiwara S (CH), Akahoshi M (CN), Suyama A (EN), Kasagi F (EH), Ozasa K (EH)

Studies of cancer risks among A-bomb survivors have mainly focused on the relationship with A-bomb radiation. However, exposure to medical radiation, such as diagnostic X-ray exposure and radiotherapy, is on the increase globally. Under these circumstances, it is important to study the effects of medical radiation exposure in the LSS population. Those who underwent cancer radiotherapy after exposure to A-bomb radiation constitute a unique population, because they have been exposed to multiple radiation insults at different times (A-bomb and medical radiation). However, the combined effects of a prior exposure followed many years later by a subsequent exposure to ionizing radiation have not been well addressed either by experimental or epidemiological studies. By determining the magnitude of cancer risks among those who were exposed to both atomic bomb and therapeutic radiation using a population-based epidemiological follow-up study, new light will be shed on how atomic-bomb radiation exposure modifies the risks of subsequent radiation exposure or vice versa.

In this study, we will follow up the (secondary) cancer incidence after radiotherapy and mortality from cancer and non-cancer diseases among 1,501 LSS subjects who were confirmed to have undergone radiotherapy based on three surveys during the 1960's to early 1980's. This study evaluates not only the effects of A-bomb radiation but also the effects of medical radiation and the combined effects of the two kinds of radiation.

The RP was approved in August, 2009. Applications for use of tumor registry data have been submitted to the Hiroshima and Nagasaki registry committees and approved in February, 2010. A database is being created containing information on primary diseases for which therapy was conducted (malignant tumor and other diseases; kind of cancer in case of malignant tumor), age and date of radiotherapy, site of radiotherapy, and estimated radiation dose to major tissues and organs from radiotherapy.

RP 1-09 A nested case-control study of factors contributing to acceleration of the development of hepatocellular carcinoma using stored sera (Addendum to RP 1-04)

Ohishi W (CH), Fujiwara S (CH), Cologne JB (S), Akahoshi M (CN), Niwa Y (R), Nishi N (EH), Suzuki G, Tsuge M, Chayama K

The hypothesis behind this study is that chronic inflammation due to radiation exposure may be involved in the development of hepatocellular carcinoma (HCC) through insulin resistance. The objective of this study is to examine the contribution of insulin resistance to HCC risk, taking into account radiation exposure, hepatitis virus infection,

lifestyle-related factors, and severity of liver fibrosis. This research protocol is an addendum to RP 1-04. The primary objective of RP 1-04 is to study the effects of radiation exposure, hepatitis virus infection, and lifestyle-related factors on the risk for development of HCC. In the previous study, we demonstrated that hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, obesity, and alcohol consumption are independent risk factors for HCC. The combination of HCV infection and increased body mass index (BMI) exerted a synergistic effect on risk of HCC (Ohishi et al., *Cancer Epidemiology, Biomarkers Prevention* 2008; 17:846–54). Furthermore, the ongoing analyses suggest that radiation exposure, alcohol consumption, and BMI are all independently associated with increased risk of non-B, non-C HCC.

This research protocol was approved in April, 2009 and we have started measurements of blood cytokines using stored sera obtained from HCC cases and controls of this nested case-control study (RP 1-04). Of 1,372 samples, we measured blood cytokine levels such as TNF-α, IL-6, MCP-1, leptin, and resistin with the ELISA method or the multiplex Luminex method for 1,146 samples.

RP 5-08 Breast cancer incidence among atomic-bomb survivors, 1950–2005

Yonehara S, Nishisaka T, Nakashima M, Furukawa K (S), Soda M (EN), Suyama A (EN), Sekine I, Tokuoka S, Mabuchi K, Preston DL, Kodama K (CS), Kasagi F (EH), Ozasa (EH)

Breast cancer is one of major cancers in which a significant excess risk has been observed in relation to radiation, but no difference in histological distribution was observed in the past between exposed and control cases. Histological reviews according to the new classification system will permit a re-evaluation. The procedures of case collection were based on the guidelines of special cancer studies (RP 9-88). A total of 1,632 probable cases of breast cancer were identified in the LSS cohort during 1950–2005, including 16 male cases. The screening using case information sheets has started, and 223 cases have been screened of which 196 cases have been accepted for histological review. After completing the histological reviews, the risk of breast cancer for radiation will be evaluated within histological classes using excess relative risk and excess absolute risk models. More detailed analyses of the major histological classes will consider, as the numbers of cases permit, the shape of the dose-response relationship and effect modification by age at exposure, attained age, menopausal status, etc. Histological characteristic of breast cancer cases that were highly exposed to radiation will be summarized by the pathologists compared with those with low/no exposure. A supplemental investigation of "intrinsic subtypes" (defined by estrogen and progesterone receptors, and human epidermal growth factor receptor-2 [HER-2] status) to be determined by immunochemical staining has been planned and an addendum RP has been drafted and is in internal review.

RP 4-07 Pathology study of malignant tumors of soft tissue and bone among A-bomb survivors, 1957–2003

Yonehara S, Hayashi T, Daimaru Y, Sekine I, Tokuoka S, Soda M (EN), Suyama A (EN), Kodama K (CS), Mabuchi K, Ron E, Preston DL, Ozasa K (EH)

The excess risk of sarcomas of the soft tissues and bones associated with high therapeutic doses of radiation has been known for some time, but epidemiological data on the risk associated with radiation exposure at relatively low doses are very limited. The latest analysis of solid cancer incidence data of the LSS cohort provided, for the first time in this cohort, evidence of a significant dose response for broadly-classified sarcomas using the tumor registry-based incidence data. We are conducting a detailed, standardized pathology review of sarcomas in order to investigate the association between estimated radiation dose (DS02) and risk of sarcomas by histological types and sub-types. Soft-tissue and bone sarcomas occurring between 1957 and 2003 in the LSS cohort will be identified based on the guidelines of special cancer studies (RP 9-88). A panel of pathologists will review the specimens using the WHO's Histological Classification of Tumors of Soft Tissue and Bone (2002). Analyses will be performed to assess the radiation-related risk of sarcomas and to evaluate modifying effects, if any, of age, gender, and other factors. At present, a total of 160 cases were accepted for histological review among 4,318 possible cases. Histological specimens are being collected and a trial of histological reviews has been started by the pathologists.

RP 1-06 Study on cancer of the uterus among A-bomb survivors in the Life Span Study cohort, 1950–2003 (Addendum to RP 8-85)

Tokuoka S, Fujihara M, Matsuo T, Nishisaka T, Nakajima H, Hirai Y (G), Soda M (EN), Suyama A (EN), Sekine I, Ron E, Preston DL, Mabuchi K, Kodama K (CS), Ozasa (EH)

Among the LSS cohort, cases of cancer of the uterus will be ascertained based on the guidelines of special cancer studies (RP 9-88). Reported cases will be reviewed by a panel of pathologists for histological diagnoses according to the WHO Histological Classification of Female Genital Organs (2003). It has been decided that the case collection will start for corpus cancer. The procedures for cervical cancer are suspended because less relationship with radiation was shown in the incidence study and investigations on HPV infection require a heavier load for collecting specimens from collaborating hospitals.

Investigations on atypical hyperplastic endometrial lesions and cervical dysplastic lesions are also suspended because case collection through the passive surveillance mechanisms such as cancer registries cannot gather those cases without biases. Those early-stage lesions are thought be detected through cancer screening or incidental findings when patients visit clinics. Hence, the incidence rates of those lesions would depend on patients' medical service-seeking behaviors, which might differ by radiation dose because the frequency of other health conditions is associated with radiation exposure. Active surveillance such as uterine cancer screening among all LSS subjects would be required to avoid such biases, but there are no such

systems. The association between estimated radiation doses (DS02) and histologically confirmed corpus cancer and its sub-types will be investigated. Collection of case records has started.

RP 2-04 A case-control study of atrophic gastritis and gastric cancer using frozen sera and genomic DNA: Identification of new biomarkers for chronic gastritis associated with gastric cancer

Fujiwara S (CH), Suzuki G, Cullings HM (S), Ohishi W (CH), Hayashi T (R), Nakachi K, Tahara E, Akahoshi M (CN)

The primary goal of this study is to determine whether the radiation exposure-dependence of gastric cancers seen in the atomic-bomb survivors is related to chronic tissue inflammation associated with *H. pylori* infection. Specific aims include (1) establishing new biomarkers for pathogenic *H. pylori* and related chronic gastritis; and (2) identifying the genetic factors controlling the host's inflammatory response to bacterial infections.

Results have indicated that *H. pylori* infection, chronic gastritis, and smoking are all independent predictors of gastric cancer. In terms of radiation-dose dependency, higher relative risks were noted with the diffuse type of gastric cancers, whereas much lower risks were noted with the intestinal type of gastric cancers, after adjusting for these risk factors. We are continuing to analyze other risk factors that might link chronic inflammatory conditions to radiation-associated gastric cancers. Results obtained from our serum marker analyses have been published (Suzuki et al., *Cancer Epidemiology, Biomarkers and Prevention* 2007; 16:1224–8). Analyses of genotypes of *IL-1B*, *LTA*, etc. have been finished. The *LTA 252* genotype is associated with noncardia gastric cancer of the diffuse type in Japan, and the genotype was an effect modifier for radiation dose (Suzuki et al., *Helicobacter* 2009; 14:571–9) (Table).

Table. Interaction among three risk factors: radiation risk for non-cardia gastric cancer is restricted to subjects with LTA 252G-carriate and smoking status

Risk categories	RR	95% CI	*p*
Radiation dose (1 Gy)	0.8	0.5–1.2	0.3
Radiation dose (1 Gy) for current smoker with LTA 252G-carriage	1.3	0.6–1.9	0.4
Radiation dose (1 Gy) for noncurrent smoker with LTA 252AA-carriage	2.0	0.6–3.4	0.2
Radiation dose (1 Gy) for noncurrent smoker with LTA 252G-carriage	3.8	1.7–5.9	0.009

(*Suzuki et al., Helicobacter 2009; 14:571–9*)

We are also analyzing the relationship between gastric cancer and chronic gastritis in relation to radiation exposure. Radiation risk was significant only for people without chronic gastritis in developing diffuse type non-cardiac gastric cancers. To confirm the results, an RP on model building for joint effects of radiation and radiation-related intermediate risk factors in nested case-control studies has been prepared by the Department of Statistics.

RP 1-04 A nested case-control study of hepatocellular carcinoma among atomic-bomb survivors using stored sera

Ohishi W (CH), Fujiwara S (CH), Cologne JB (S), Suzuki G, Akahoshi M (CN), Nishi N (EH), Chayama K

The primary objective of this study is to investigate the relationship between radiation exposure and the risk of hepatocellular carcinoma (HCC) among A-bomb survivors after taking into account hepatitis virus infection. Our working hypothesis is that radiation exposure accelerated HCC occurrence in the early stage of liver fibrosis after hepatitis C virus (HCV) infection. The study includes evaluations of the interactions between (1) the initial dose of radiation; (2) the status of hepatitis virus infection; and (3) the severity of liver fibrosis, along with other potential risk factors to be evaluated in terms of the etiology of HCC. For this purpose, among cases and controls, selected biomarkers of evolving liver disease will be assayed, including hepatitis virus markers and fibrosis markers. Currently, there are stored sera from some 224 HCC cases and three controls per case matched on age, sex, city, and time of stored sera, and countermatched on radiation exposure. Hepatitis B virus (HBV) and HCV infection, alcohol consumption and body mass index (BMI) of >25 kg/m^2 (obesity) 10 years before HCC diagnosis were independent risk factors that contributed to increased HCC risk. HBV and HCV infection and obesity remained independent risk factors after adjusting for severity of liver fibrosis. Results have been published (Ohishi et al., *Cancer Epidemiology, Biomarkers and Prevention* 2008; 17:846–54).

We estimated relative risks (RR) of HCC for HBV or HCV infection and excess RR (ERR) of HCC for liver dose of radiation. After adjusting for alcohol consumption and BMI, the ERR per Gy (ERR/Gy) of radiation exposure for HCC was 0.55 ($P = 0.003$), while the RRs for HBV or HCV infection were 61 ($P < 0.001$) and 80 ($P < 0.001$), respectively. These estimates changed little when radiation and viral effects were fit jointly. The ERR/Gy of radiation exposure for non-B, non-C HCC was 1.15 ($P = 0.026$) with adjustment for alcohol consumption or BMI. These results indicated that HBV and HCV infection and radiation exposure are associated independently with increased risk of HCC, and that radiation exposure is a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption or BMI.

RP 6-02 A nested case-control study of breast and endometrial cancer in the cohort of Japanese atomic bomb survivors

Neriishi K (CH), Sharp GB, Eguchi H, Nakachi K, Cologne JB (S), Nakashima E (S), Izumi S, Grant EJ (EH), Fujiwara S (CH), Akahoshi M (CN), Key TJ, Stevens RG, Kabuto M, Land CE

The purpose of this study of breast and endometrial cancer etiology is to characterize the joint effects of radiation and serum-based indicators of hormonal status, oxidative stress and phytoestrogen consumption.

The research protocol had examined a series of hormone-related serum measurements, including total estradiol (E2), free E2, testosterone, sex hormone binding globulin (SHBG), progesterone, insulin-like growth factor-1

(IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and prolactin. The study also included genistein measurements to index phytoestrogen consumption, and biomarkers indicative of anti-oxidant availability and iron-mediated oxidative stress, D-Rom and ferritin, respectively. This is the first study to simultaneously analyze such a wide variety of blood serum components in relation to breast or endometrial cancer, and is one of the few to utilize blood samples collected up to 30 years before cancer diagnosis. Controls were selected by counter-matching on radiation dose so as to increase the statistical power to detect radiation effects.

All the laboratory measurements have been completed on the 243 breast cancer cases and 486 matched controls. Two manuscripts on factors influencing hormone levels on controls have been completed in 2009, and the results revealed a significant radiation dose-dependent increase in estrogen and testosterone levels among postmenopausal women. The manuscripts will soon be submitted for review and publication. In 2010, appropriate analytical methods for the joint relationship of radiation and hormones upon cancer risk will be developed, with which the data will be analyzed.

RP 2-91 Studies on skin cancer incidence among the RERF Extended Life Span Study cohort, Hiroshima and Nagasaki, 1950–87
RP 2-02 Studies on skin cancer incidence among the RERF Life Span Study cohort, Hiroshima and Nagasaki (Addendum to RP 2-91)

Tokuoka S, Kishikawa M, Iseki M, Yonehara S, Soda M (EN), Mabuchi K, Ron E, Preston DL, Sugiyama H (EH), Misumi M (S), Suyama A (EN), Ozasa K (EH), Kodama K (CS)

It has been reported that skin cancer incidence, especially nonmelanoma skin cancer, is increased by radiation among patients with radiotherapy and atomic-bomb survivors, and also reported that the effects of radiation upon skin cancer have a long latency period among atomic-bomb survivors. This study (RP 2-02) extends the skin cancer ascertainment from 1987 to1996. Histological review of new cases has been completed. The total number of cases is 700 and 336 first primary skin cancer cases were identified among them. By histological type, the following cases were observed: Malignant melanoma (n = 10), basal cell carcinoma (n = 123), squamous cell carcinoma (n = 144), Bowen's disease (n = 64), Paget's disease (n = 10), and other skin cancer cases (n = 15). ERRs were estimated assuming a linear dose response, and only basal cell carcinoma had a statistically significant positive dose response (ERR/Gy = 2.1, 95% confidence interval [CI] = 1.2–3.5, $P < 0.01$) (Figure), the previous study with follow-up through 1987 reported an ERR/Gy of 1.8 (90% CI = 0.83–3.3). Other models were also explored in an effort to find a best fit model using Akaike information criteria (AIC). The best fit dose-response model estimated a dose threshold at 0.6 Gy (95% CI = 0.34–0.89), an ERR/Gy of 2.7 (95% CI = 1.1–5.1). This model predicted an ERR at 1 Gy of 1.1 (95% CI = 0.43–2.05). In conclusion, epidermal basal cells are sensitive to ionizing radiation, especially for people who were young at exposure. The threshold for a radiation dose response of basal cell carcinoma was estimated to be 0.6 Gy, lower than the 1.0 Gy reported in the previous study. A manuscript is in preparation and will be submitted in 2010.

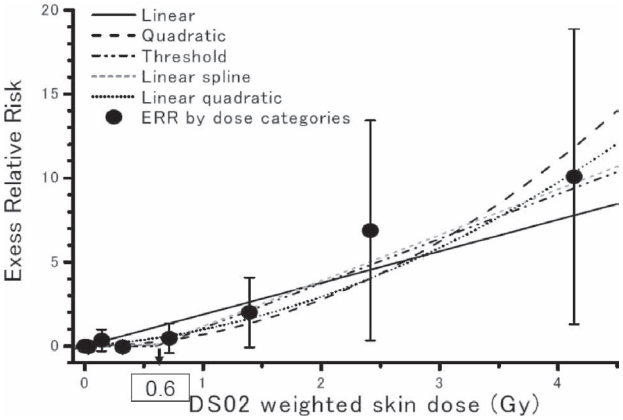


Figure. Risk of incidence of basal cell carcinoma of skin by dose in various models for dose-response relationship. The models include sex, period at diagnosis, log attained age, and AHS membership as background parameters, and age at exposure as an effect modifier. Closed circles are point estimates of excess relative risk for specific dose category and whiskers are 95% confidence intervals.

RP 3-94 Incidence of lymphoid malignancies among the atomic-bomb survivors, 1950–90

Tokuoka S, Namba K, Fujihara M, Tokunaga M, Takahara O, Soda M (EN), Dohy H, Kamada N, Tomonaga M, Preston DL, Mabuchi K, Kodama K (CS)

The relationship between lymphoid malignancies and radiation exposure is complicated because of variability of diagnostic categories and uncertainty about the potential for radiation carcinogenesis for certain important categories. On the other hand, recent advances in immunological studies of malignant lymphoma (ML) are remarkable. Surface markers on tumor cells can be used to distinguish B and T cells involved in malignant lymphomas, and a new WHO classification scheme has replaced earlier classifications of lymphomas.

The purpose of this study is to characterize the LSS sample in terms of risk for the broad spectrum of lymphoid malignancies by cell type in relation to radiation dose from the atomic bombings and other factors. Emphasis will be placed on extensive case ascertainment, and confirmation and classification of cases by standardized pathology review. Lymphoma cases are being classified according to the WHO classification (published in 2001) using immunohistochemical studies into T-ML, B-ML, Hodgkin lymphoma, or others. Further subtypes of B-ML are also being coded. Diagnosis of adult T-cell leukemia/lymphoma (ATL) is based on detection of proviral DNA of HTLV-I using polymerase chain reaction (PCR) and other current technology. The nested classification system by InterLymph will be applied to the confirmed cases. The procedures of case collection were based on the guidelines of special cancer studies (RP 9-88).

Histopathological reviews identified 468 cases of ML, including 303 cases of B-cell origin (129 cases of diffuse large B-cell lymphoma), 131 cases of T-cell origin, and 13

cases of Hodgkin's lymphoma. Reviews for lymphocytic leukemia were performed through registries records such as HE39, FAB, and tumor registries, and some controversial cases have been histologically reviewed and final diagnoses are being determined.

RP 1-94 Studies of lung cancer incidence among the atomic-bomb survivors, 1950–90

Tokuoka S, Kasagi F (EH), Egawa H, Matsuo T, Yonehara S, Nakashima E (S), Furukawa (S), Soda M (EN), Tokunaga M, Mabuchi K, Preston DL, Ozasa K (EH), Kodama K (CS)

Lung cancer is a well established late effect of radiation exposure in various irradiated populations, including the atomic-bomb survivors. The incidence analysis of the RERF tumor registry data for the period 1958–98 also observed a radiation-related increase in lung cancer risk. The ERR for radiation effects tends to increase with increasing age at exposure, while attained-age specific EAR estimates vary little with age at exposure. These patterns are the opposite of those seen for solid cancers as a group. However, several specific issues and questions remain, including the specificity of various cell types involved in radiation- versus smoking-related cancers, confounding and joint effects of smoking in relation to radiation exposure, delineation of temporal trends with allowance made for age at exposure and attained-age effects. This RP was developed to address those questions and issues. The study is evaluating lung cancer incidence in the Life Span Study (LSS) for the period 1950–99. The pathologist panel has histologically confirmed 2,368 lung cancers in LSS participants among the 5,711 potential cases that were ascertained from autopsy, surgical pathology records, and death certificates, as well as from the tumor and tissue registries and major medical institutions in Hiroshima and Nagasaki. These tumors were classified according to the WHO 1999 histological classification scheme. Three papers are in preparation: Radiation risk by histological types of lung cancer, temporal trends for histological types of lung cancer, and smoking-related lung cancer risk by histological type.

A paper on the joint effects of smoking and radiation on lung cancer was accepted for publication in *Radiation Research* written by Dr. Furukawa. The joint effect appeared to be dependent on smoking intensity, and was best described by a generalized interaction model rather than simple additive or multiplicative models. Specifically, there appears to be a multiplicative association of smoking and radiation at lower smoking-intensity levels, but no joint effect at high smoking-intensity levels. Another paper on smoking and radiation effects on various histological types of lung cancer is ready for submission.

RP 2-92 Studies of ovarian tumor incidence among the RERF Extended Life Span Study cohort, 1950–87

Tokuoka S, Kawai K, Inai K, Shimizu Y (EH), Nakashima E (S), Tokunaga M, Soda M (EN), Mabuchi K, Kodama K (CS)

An increased risk of ovarian cancer among atomic-bomb survivors has been reported from an earlier site-specific study of ovarian cancer as well as from analyses of LSS mortality and incidence. No clear evidence exists for any specific histologic type being particularly associated with radiation exposure. The aim of this study is to examine and quantify the relationship between the development of malignant and benign ovarian tumors and exposure to atomic-bomb radiation in the LSS based on histologically confirmed cases. The present study extends the previous ovarian cancer series by seven years. A total of 601 ovarian tumors (182 malignant, 419 benign tumors) were histologically confirmed. Frequent histological types were "serous epithelial tumor" (48% for malignant and 37% for benign tumors), "mucinous epithelial tumors" (22% and 19%, respectively), "sex-cord stromal tumors" (7% and 12%, respectively), and "germ cell tumors" (3% and 28%, respectively), which was similar to other studies. There was a suggestion of variation in histological types of ovarian tumors in relation to radiation dose within the case series. The mucinous type seems to be less radiogenic than the other types. There was a significant apparent survival advantage for the mucinous type compared with the serous type. We confirmed this suggestion in the population-based incidence study. Within tumor types, there were no consistent differences in survival by radiation dose. "Malignant and benign ovarian tumors among atomic-bomb survivors in Hiroshima and Nagasaki" was presented at the 16th International Meeting of the European Society of Gynaecological Oncology, October, 2009.

RP 6-91 Studies of thyroid tumor incidence among the RERF Extended Life Span Study cohort, 1950–87

Tokuoka S, Hayashi Y, Tsuda N, Tokunaga M, Furukawa K (S), Sakata R (EH), Ron E, Mabuchi K, Lagarde F, Kodama K (CS)

Thyroid cancer was one of the earliest solid cancers found to be increased in atomic-bomb survivors, and several thyroid cancer studies have been conducted among survivors over the last 40 years. This study includes the 1958–1995 period of case ascertainment for both benign and malignant thyroid tumor cases. Extending the study period to 37 years permits more precise characterization of the shape of the dose-response relationship for malignant and benign tumors, temporal patterns and risk modifiers, and the relative importance of different histological subtypes to radiation exposure. Tumors are ascertained from the Tumor and Tissue Registries in Hiroshima and Nagasaki as well as from autopsy, surgical record files, and death certificates maintained by RERF and other major medical institutions. A uniform classification system is used for the pathology review (Hedinger et al., *International Classification of Tumors, Histological Typing of Thyroid Tumors [2nd ed]*. Berlin: Springer-Verlag; 1988). All the work related to case ascertainment and histological grouping has been completed. A total of 2,903 potential cases were reviewed, from which 1,074 thyroid tumor cases were identified. Histological verification was possible for 1,036 (96%) of these cases, 697 with malignant tumors and 339 with benign tumors. Most the malignant thyroid carcinomas were of the papillary type (95%), followed by the follicular type (2%), malignant lymphoma (1%), medullary (<1%), and undifferentiated carcinoma (1%). The 663 papillary carcinomas included 325 micro-carcinomas detected primarily at autopsy. A pathology paper by Dr. Hayashi et al., entitled "Papillary microcarcinoma

of the thyroid among atomic-bomb survivors: Tumor characteristics and radiation risk," has been published (*Cancer* 2010; 116:1646–55). It describes the histological distribution of thyroid tumors and the characteristics and radiation risk of papillary microcarcinoma among A-bomb survivors, and emphasizes how exposure to low-to-moderate doses of ionizing radiation appears to increase the risk of thyroid papillary microcarcinoma, even when exposure occurs during adulthood.

RP 9-88 Guidelines for the conduct of site-specific cancer incidence studies among A-bomb survivors, Hiroshima and Nagasaki

Tokuoka S, Sekine I, Soda M (EN), Suyama A (EN), Kasagi F (EH), Kodama K (CS), Tokunaga M, Mabuchi K, Cullings HM (S), Ozasa (EH)

These guidelines are intended to simplify the preparation of research plans for site-specific cancer incidence studies and provide uniformity in basic study design and operation. The guidelines state that case ascertainment would be undertaken through the Hiroshima and Nagasaki tumor registries supplemented by an extended case-finding search. A panel of collaborating pathologists specializing in pertinent areas would achieve agreement on histopathological classification and verification. Tumor types are to be classified using internationally accepted tumor classification systems. RERF and/or other participating epidemiologists and statisticians should perform data analyses. Under these guidelines, the following site-specific cancer incidence studies are currently being undertaken: Skin cancer (RPs 2-91 and 2-02), thyroid tumor (RP 6-91), breast cancer (RP 5-08), ovary cancer (RP 2-92), uterine cancer (both cervical and endometrial) (RP 1-06), lung cancer (RP 1-94), soft tissue and bone tumors (RP 4-07), and lymphoid malignancies (RP 3-94). Papers on thyroid microcarcinoma and lung cancer have been accepted in *Cancer* and *Radiation Research*, respectively. The majority of the current site-specific studies are expected to be completed in the next three to four years.

RP 2-86 Collection of surgically removed cancer tissues from A-bomb survivors: Special reference to thyroid and breast cancers

Hamatani K (R), Neriishi K (CH), Taga M (R), Eguchi H, Imai K (R), Nakachi K

This protocol is concerned with the collection and cryopreservation in liquid nitrogen of possibly radiation-induced fresh thyroid and breast cancer tissues to be used as a resource for future molecular oncology studies. However, despite our continuing interest, we have found it extremely difficult to collect either fresh or archival tissues from the LSS cohort. Plans are being worked on to provide better access to surgical tissues from cancer patients in Hiroshima and Nagasaki.

RP 29-60 Detection of leukemia and related disorders

Soda M (EN), Sugiyama H (EH), Kodama K (CS), Suyama A (EN), Ozasa K (EH), Tomonaga M, Kimura A, Kamada N, Dohy H, Iwanaga M, Miyazaki Y, Hsu WL (S), Cologne JB (S)

This case-finding program, known as the leukemia registry, was started in 1948, with the collaboration of hematologists and physicians involved in the diagnosis and treatment of leukemia patients in Hiroshima and Nagasaki. With the recent improvement in the Hiroshima and Nagasaki tumor registries, leukemia case ascertainment currently relies primarily on the tumor registries' activities. In the mid-1980s, more than 60% of leukemia cases in the leukemia registry were reclassified using modern diagnostic criteria and nomenclature, most notably the French-American-British (FAB) classification schema for the acute leukemias.

Myelodysplastic syndrome (MDS) is the target of a new study conducted in Nagasaki (Soda, Tomonaga, and Iwanaga). From among 68 MDS cases identified, 47 had known DS02 doses. Analyses of the association between MDS incidence and radiation dose were conducted in collaboration with the Department of Statistics. An apparent radiation dose-response relationship in the risk of MDS was observed (Hsu) (Figure). MDS risk showed a significant linear dose response. The excess relative risk (ERR) at 1 Gy was 4.27 (95% CI, 1.63–9.48). A paper was prepared and submitted to an international medical journal.

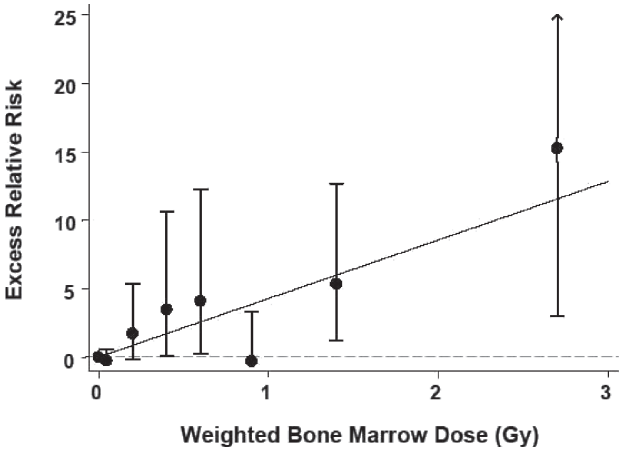


Figure. Radiation Dose-response for MDS. The bold line is the fitted linear excess relative risk (ERR) dose response without risk modification. The closed circles indicate point estimates of sex-averaged ERR for specific dose category, and whiskers show the 95% confidence intervals. The sex-averaged ERR at 1Gy was 4.27 (95% CI, 1.63–9.48). The dashed horizontal line represents ERR = 0.

Comprehensive incidence analyses for leukemia and related diseases are being conducted in collaboration with the Department of Statistics. The collection of cases of hematological malignancies is being continued through the city and prefecture cancer registries of the two regions, as before.

RP-A12-08 Second collaborative analysis of radiation-associated thyroid cancers

Sakata R (EH), Ron E, Veiga L, Lubin J, Sugiyama H (EH), Shore RE (D)

The purpose of this project is to improve our understanding of radiogenic thyroid cancer by conducting an updated and expanded pooled analysis of 16 studies that have a reasonable number of thyroid cancers in exposed individuals and individual thyroid dose estimates. While the

results from the previous pooling study of thyroid cancer and radiation risk remain the most important summary of epidemiologic information on thyroid cancer risks from exposure to external radiation to date, questions remain regarding the risk associated with adult exposure, the shape of the dose-response curve at low and high doses, the effect of fractionated dose, the risk associated with specific thyroid cancer histological types, the effect of gender, and the relationship with attained age and time since exposure. Since the prior pooling study (Ron et al., *Radiation Research* 1995; 141:259–77), a considerable amount of new data has been generated, so a new pooled analysis will significantly add to what is known about radiation-related thyroid cancer.

The general approach of this collaborative study is to examine how the age-specific thyroid tumor risks depend on variables of interest. To carry out these analyses, data are cross-classified by age (or age of exposure), calendar period, study population, dose, and other variables. For each cell, the number of events and accumulated person years are recorded and the person-year weighted mean radiation doses calculated. The disease rates are then analyzed using Poisson regression.

This RP was approved in October 2008. Data have been sent to the US National Cancer Institute where the pooled data are being analyzed.

RP-A5-08 Esophageal and gastric cancers: Patterns and predictors of risk in Hiroshima and Nagasaki, Japan

Kennedy BS, Mabuchi K, Chow WH, Kasagi F (EH), Suyama A (EN), Shimizu Y (EH), Sugiyama H (EH), Soda M (EN), Sakata R (EH), Grant EJ (EH), Cologne JB (S), Cullings HM (S), Yamada M (CH)

The patterns and predictors of esophageal and gastric cancer risk are being examined within the Hiroshima and Nagasaki populations using several approaches. First, the temporal trends in age-adjusted incidence rates of esophageal and gastric cancers among males and females are being investigated using the tumor registries for Hiroshima and Nagasaki. Trends will be assessed by anatomic location as well as by histological cell type. Second, the effects of ionizing radiation on the risk of esophageal and gastric cancers among atomic-bomb survivors are being studied using the extended Life Span Study (LSS) population. Similar to the temporal trend analyses, this component of the study will examine the association of radiation dose with esophageal and gastric cancer risk by anatomic location and histological cell type, while controlling for potential confounders (e.g., age of exposure, attained age, time since exposure). Third, using supplemental information gathered on the atomic-bomb survivors, the predictors of esophageal and gastric cancers are being investigated, with an emphasis on potentially modifiable risk factors (e.g., tobacco smoking, alcohol consumption, body mass index, dietary patterns). Further, the population attributable risk (PAR) for these factors will be estimated in order to quantify their public health impact.

Dr. Kennedy visited RERF as a Beebe fellow between January and June of 2008. At the end of his visit, Dr. Kennedy presented preliminary results of his research. He tracked the gastric cancer and esophageal trends in the Hiroshima population-based registry and estimated the radiation risks of specific sub-types of esophageal cancers. Additional analyses and a manuscript are in preparation. A manuscript is due in 2010.

Special Cancer Studies Publications

RERF Report (RR)

♦ Suzuki G, Cullings HM, Fujiwara S, Matsuura S, Kishi T, Ohishi W, Akahoshi M, Hayashi T, Tahara E: *LTA 252GG* and *GA* genotypes are associated with diffuse-type noncardia gastric cancer risk in the Japanese population. Helicobacter 2009 (December); 14(6):571–9.

 (RR 1-09)

[Abstract] ***Background.*** There are limited numbers of reports on the association of *lymphotoxin-alpha (LTA)* genotypes with gastric cancer. ***Methods.*** A nested case-control study was carried out in the longitudinal cohort of atomic bomb survivors using stored sera before diagnosis (mean, 2.3 years) and blood cells. Enrolled were 287 cases with noncardia gastric cancer of diffuse and intestinal types and three controls per case selected from cohort members matched on age, gender, city, and time and type of serum storage and counter-matched on radiation dose. ***Results.*** *LTA 252GG* and *GA* genotypes were associated with the prevalence of *Helicobacter pylori* IgG seropositivity and higher antibody titer against *H. pylori* cytotoxin-associated gene A (CagA) protein in controls and they were an independent risk factor for noncardia gastric cancer of diffuse type (RR = 2.8 (95% CI: 1.3–6.3), $p = .01$, and RR = 2.7 (95% CI: 1.5–4.8), $p < .001$), but not for intestinal type, after adjusting for *H. pylori* IgG seropositivity, CagA antibody titers, chronic atrophic gastritis, smoking, and radiation dose. Cessation of smoking (RR = 0.4 (95% CI: 0.2–0.7), $p < .001$) and never smoking (RR = 0.4 (95% CI: 0.3–0.6), $p < .001$) were both protective for future noncardia gastric cancer. Radiation dose was associated with noncardia gastric cancer in subjects with both the *LTA 252G*-allele and never smoking/quit smoking histories (RR = 3.8 (95% CI: 1.7–5.9), $p = .009$). ***Conclusions.*** The *LTA 252* genotype is associated with noncardia gastric cancer of diffuse type in Japan and interacted with radiation dose.

Manuscripts in Press

⌘ Furukawa K, Preston DL, Lönn S, Funamoto S, Yonehara S, Matsuo T, Egawa H, Tokuoka S, Ozasa K, Kasagi F, Kodama K, Mabuchi K: Radiation and Smoking Effects on Lung Cancer Incidence among Atomic Bomb Survivors. Radiation Research.

⌘ Hayashi Y, Lagarde F, Tsuda N, Funamoto S, Preston DL, Koyama K, Mabuchi K, Ron E, Kodama K, Tokuoka S: Papillary microcarcinoma of the thyroid among atomic bomb survivors. Tumor characteristics and radiation risk. Cancer.

Special Cancer Studies Oral Presentations

❖ Furukawa K, Lönn S, Funamoto S, Mabuchi K, Egawa H, Tokuoka S, Preston DL. Interaction effects of radiation and smoking on lung cancer risks among atomic-bomb survivors. Late Health Effects of Ionizing Radiation, 4–6

May 2009, Washington DC, USA (related to *Tumor and Tissue Registry*)

❖ Shimizu Y, Inai K, Kawai K, Tokunaga M, Soda M, Mabuchi K, Land CE, Tokuoka S. Malignant and benign ovarian tumors among atomic-bomb survivors in Hiroshima and Nagasaki. The 16th International Meeting of the European Society of Gynaecological Oncology, 11–14 October 2009, Belgrade, Serbia

❖ Sugiyama H, Misumi M, Kishikawa M, Iseki M, Yonehara S, Hayashi T, Nishi N, Soda M, Tokuoka S, Shimizu Y, Sakata R, Grant EJ, Mabuchi K, Kasagi F, Suyama A, Ozasa K. Skin cancer incidence among Life Span Study cohort, Hiroshima and Nagasaki (1958–1996). The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima

❖ Grant EJ, Neriishi K, Cologne JB, Eguchi H, Hayashi T, Geyer SM, Izumi S, Nishi N, Land CE, Stevens RG, Sharp GB, Nakachi K. Effects of radiation on serum risk markers of breast cancer found in healthy A-bomb survivors. The Joint Scientific Meeting of IEA Western Pacific Region and the 20th Japan Epidemiological Association, 9–10 January 2010, Koshigaya

❖ Ohishi W, Fujiwara S, Cologne JB, Akahoshi M, Niwa Y, Suzuki G, Tsuge M, Chayama K. Effect of radiation exposure on risk of hepatocellular carcinoma in atomic-bomb survivors. The 8th American Association for Cancer Research/Japanese Cancer Association Joint Conference, 5–9 February 2010, Waikoloa, Hawaii, USA

Research Protocols 4-08, 18-61

Tumor and Tissue Registries, Hiroshima and Nagasaki

Note that RERF studies related to the tumor and tissue registries include RPs 2-09, 1-09, 5-08, 4-07, 1-06, 2-04, 1-04, 6-02, 2-02, 3-94, 1-94, 2-92, 6-91, 2-91, 9-88, 2-86, 29-60, A12-08, and A5-08 (all discussed under Special Cancer Studies*).*

RP 4-08 Investigation of storage conditions for cataract tissue of A-bomb survivors, and its collection and storage program

Neriishi K (CH), Blakely EA, Chang P, Nakashima E (S), Ohishi W (CH), Fujiwara S (CH), Hida A (CN), Akahoshi M (CN), Hayashi T (R), Ito R (R), Nakachi K, Minamoto A, Yokoyama T, Toda S, Uematsu M, Tsuiki E, Kiuchi Y, Kitaoka T, Shirai A, Cucinotta FA, Chylack LT

The goal of this project is to confirm the adequacy of a storage method for cataract tissues of AHS participants who undergo a cataract operation, and to collect and store the tissue for future analyses. Our recent study has indicated that the odds ratio at 1 Sv of prevalence of those who underwent cataract operation was 1.39 (95% confidence interval: 1.24, 1.55). AHS participants who were young at the time of the bombings are expected to reach the age of cataract operation within the next decades. The stored cataract tissues are expected to contribute significantly to future research on radiation-induced cataract. When enough numbers of tissues are collected, a new research protocol for biological studies will be prepared.

Meetings with specialists were held to establish the adequacy of the storage method for cataract tissues of the AHS participants. Training of staff for collection and storage of the tissue for future analyses was also conducted. Based on the established method, actual program of collection and storage of lens tissue has started on January 2009 in Hiroshima and 20 samples have been collected and stored as of March 2010. The same program was more recently begun in Nagasaki.

RP 18-61 Tumor registry study in Hiroshima and Nagasaki

Soda M (EN), Sugiyama H (EH), Grant EJ (EH), Suyama A (EN), Kasagi F (EH), Shimizu Y (EH), Katayama H (IT), Ozasa K (EH), Kodama K (CS)

This research protocol constitutes the framework for the operation of the Hiroshima and Nagasaki tumor registries, which provide for the systematic collection and management of tumor data in the populations of the cities and prefectures of Hiroshima and Nagasaki. These registries had been undertaken under the auspices of each city's medical association with technical and managerial support provided by RERF in Hiroshima since 1957 and in Nagasaki since 1958. In 2005 the Hiroshima cancer registries were placed under the auspices of the respective city and prefecture governments to be in compliance with the Personal Information Protection Law. Case collection by notifications and death certificates has been almost completed through 2008 in both Hiroshima and Nagasaki. On-site record abstraction is nearly complete through 2003

in Hiroshima and through 2006 in Nagasaki.

The registries are linked with the master list of the members of RERF's major RERF cohort samples (LSS, *in utero*, and F_1) and thus serve as the source of RERF cancer incidence data. Case ascertainment and data collection are based on the abstraction of medical records conducted by trained personnel at regularly scheduled hospital visits. This active approach produces high quality incidence data that are not typically seen in other tumor registries in Japan, most of which rely on passive case notifications by physicians. The Hiroshima and Nagasaki tumor registry data have regularly been included in volumes and website of "Cancer Incidence in Five Continents," compiled by the International Association of Cancer Registries (IACR) and the International Agency for Research on Cancer (IARC) in Lyon, France, and are the only Japanese population-based tumor registries accepted as Group A (highest quality) registries by the IACR/IARC. The population-based tumor registries in both Hiroshima and Nagasaki will continue to provide up-to-date cancer incidence and other information for the local communities.

No RP Number Tissue registry study

Soda M (EN), Sugiyama H (EH), Grant EJ (EH), Suyama A (EN), Kasagi F (EH), Shimizu Y (EH), Katayama H (IT), Ozasa K (EH), Kodama K (CS)

Tissue registries to collect and archive tumor tissue samples for pathological studies etc. were started in 1973 under the auspices of Hiroshima Prefectural Medical Association and in 1974 by Nagasaki City Medical Association. These were placed under the prefecture governments in recent years to be in compliance with the Personal Information Protection Law. Pathology slides and pathology reports are collected for each tumor, malignant and benign. Tissue registry and tumor registry data from each city are processed at RERF, and crosschecked with the RERF's study population with permission of each registry. The tissue registries provide a supplemental source for tumor case ascertainment for the tumor registries and were especially valuable in the early years, when there was difficulty in seeking collaboration from certain hospitals in Hiroshima. A large number of pathology slides collected and stored at one location greatly facilitates the conduct of our many site-specific studies involving pathology reviews of cases diagnosed over many years. The Hiroshima and Nagasaki tissue registries will continue to provide updated biosample data that serve as the basis for a series of LSS site-specific cancer incidence and pathology studies discussed in RP 9-88. Tissue diagnoses and samples are currently being updated through 2006. The Department of Epidemiology is linking tissue registry information with RERF subjects through 2003.

Tumor and Tissue Registry Publications

RERF Report (RR)

♦ Sugiyama H, Nishi N, Kuwabara M, Ninomiya M, Arita K, Yasui W, Kasagi F, Kodama K: Incidence and survival of childhood cancer cases diagnosed between 1998 and 2000 in Hiroshima City, Japan. Asian Pacific Journal of Cancer Prevention 2009 (October-December); 10(4):675–80. (*This abstract was reprinted by permission of Asian Pacific Organization for Cancer Prevention.*) (RR 6-09)

[Abstract] There have been few studies on cancer incidence and survival among children in Japan. Childhood cancer cases in Hiroshima City can be ascertained almost perfectly in terms of completeness and validity as both a population-based cancer registry and a tissue registry cover the whole area. We report here recent incidence and survival of childhood cancer in Hiroshima City. Subjects were cancer patients less than 15 years of age in Hiroshima City registered in the Hiroshima City Cancer Registry and/or the Hiroshima Prefecture Tumor Registry (tissue registry) between 1998 and 2000. Cancer incidence in Hiroshima City was calculated for 12 diagnostic groups according to the International Classification of Childhood Cancer, and compared with general incidence in Japan. Five-year survival was calculated by the Kaplan-Meier method. There were 63 children who had a cancer newly diagnosed during 1998–2000, with only one death-certificate-only case (1.6%). Agestandardized incidence rates (per million) was 144.3 for boys and 93.9 for girls. Leukemia was the most frequent (29%) among the 12 diagnostic groups. There were 13 cancer deaths during this period and five-year survival was 79% (95% Confidence Interval: 67%–87%). Childhood cancer incidence was slightly higher than that for all of Japan, but the relative distribution of patients by diagnostic group was compatible with the general pattern. Both of these observations might be due to the high quality of the tumor and tissue registries.

Other Journal Publications

♦ Arisawa K, Soda M, Ono M, Uemura H, Hiyoshi M, Suyama A: Letter to the Editor: Trends of incidence rate of adult T-cell leukemia/lymphoma in an HTLV-1 endemic area in Japan. International Journal of Cancer 2009 (August); 125(3):737–8.

♦ Hsu WL, Soda M, Nishi N, Preston DL, Funamoto S, Tomonaga M, Iwanaga M, Suyama A, Kasagi F: Leukemia, lymphoma, and multiple myeloma incidence in the LSS cohort: 1950-2001. Nakashima M et al. eds. Radiation Health Risk Sciences. Proceedings of the First International Symposium of the Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." New York: Springer; 2009, pp 69–73.

♦ Miura S, Nakashima M, Kondo H, Ito M, Meirmanov S, Hayashi T, Soda M, Sekine I: Significance of oncogene amplifications in breast cancer in atomic bomb survivors: Associations with radiation exposure and histological grade. Nakashima M et al. eds. Radiation Health Risk Sciences. Proceedings of the First International Symposium of the Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." New York: Springer; 2009, pp 285–93.

♦ Sugiyama H, Nishi N, Ito K, Narahara H, Yasui W, Kajihara H, Kamada N, Arita K, Ozasa K: Achievements of follow-back survey of the Hiroshima Prefecture cancer registry. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2009 (November); 62(11):528–32. (Japanese)

Manuscripts in Press

⌘ Koga Y, Iwanaga M, Soda M, Inokuchi N, Sasaki D, Hasegawa H, Yanagihara K, Yamaguchi K, Kamihira S, Yamada Y: Trends in HTLV-1 prevalence and incidence of adult T-cell leukemia/lymphoma in Nagasaki, Japan. Journal of Medical Virology.

⌘ Kondo H, Nakashima M, Soda M, Mine M, Yokota K, Shibata Y, Sekine I: Association of exposure distance with multiplicity of colorectal adenomas in Nagasaki atomic bomb survivors. Hiroshima Igaku [Journal of the Hiroshima Medical Association] (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

Tumor and Tissue Registries Oral Presentations

❖ Furukawa K, Lönn S, Funamoto S, Mabuchi K, Egawa H, Tokuoka S, Preston DL. Interaction effects of radiation and smoking on lung cancer risks among atomic-bomb survivors. Late Health Effects of Ionizing Radiation, 4–6 May 2009, Washington DC, USA (related to *Special Cancer Studies*)

❖ Nishi N, Sugiyama H, Ozasa K, Katayama H, Kodama K. Data quality of Hiroshima Prefecture cancer registry during its first three years. The 31st Annual Meeting of International Association of Cancer Registries, 3–5 June 2009, New Orleans, Louisiana, USA

❖ Soda M, Iwanaga M, Miyazaki Y, Yamada Y, Arisawa K, Sekine I, Suyama A. Trend of ATL/ATLL incidence in an HTLV-1 endemic area in Nagasaki, Japan. The 31st Annual Meeting of the International Association of Cancer Registries, 3–5 June 2009, New Orleans, Louisiana, USA

❖ Nishi N, Sugiyama H, Cullings HM, Ozasa K. Relationship between percentage of low income households and cancer mortality in mesh statistics. The 32nd Annual Meeting of the Japanese Society of Cancer Epidemiology, 16–17 June 2009, Nagoya

❖ Soda M, Iwanaga M, Miyazaki Y, Yamada Y, Arisawa K, Sekine I, Suyama A. Trend analysis of ATL/ATLL incidence in an HTLV-1 endemic area in Nagasaki. The 2nd Joint Conference of the Association for HTLV and related diseases, 29–30 August 2009, Tokyo

❖ Nishi N, Sugiyama H, Ozasa K, Kodama K, Kiya K, Hiramatsu K, Funakoshi A, Okuno H. Introduction of Hiroshima City cancer registry project. The 18th Annual Meeting of the Japanese Association of Cancer Registries, 3–4 September 2009, Niigata

❖ Soda M. Standardization and unique characteristics of population based cancer registries. The 18th Annual Meeting of the Japanese Association of Cancer Registries, 3–4 September 2009, Niigata

❖ Sugiyama H, Nishi N, Arita K, Yasui W, Kajihara H, Kamada N, Ozasa K. Cancer incidence in Hiroshima Prefecture. The 18th Annual Meeting on the Japanese Association of Cancer Registries, 3–4 September 2009, Niigata

❖ Yamada T, Nagayoshi A, Yamakawa S, Hayama S, Yoshida M, Soejima M, Soda M, Suyama A. Nagasaki Prefectural cancer registry data. The 18th Annual Meeting on the Japanese Association of Cancer Registries, 3–4 September 2009, Niigata

❖ Samartzis D, Nishi N, Cologne JB, Hayashi M, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F. The association of low to moderately high-levels of ionizing radiation exposure and the development of soft tissue sarcomas. The 29th Annual Congress of the Hong Kong Orthopaedic Association, 28–29 November 2009, Hong Kong, China (related to *Life Span Study*)

❖ Shore RE. Risk of solid cancers in children exposed to the atomic-bombs. Kids Workshop 2009 in National Institute of Radiological Sciences, 14–18 December 2009, Chiba (related to *Life Span Study*)

Research Protocols 3-04, 1-92, 10-86, 18-59
Atomic-bomb Dosimetry Studies

RP 3-04 ESR measurements of tooth samples from Nagasaki survivors (Addendum to RP 1-92)

Hirai Y (G), Nakamura N (CS), Kodama Y (G), Tomonaga M, Iijima Y, Mine M, Okumura Y, Kodama K (CS), Cullings HM (S), Akahoshi M (CN)

The purpose of the study is to investigate the possibility that Nagasaki survivors who were exposed in factories have overestimated doses. In addition to the tooth samples that were collected by the Nagasaki University group, we have newly measured 10 molars among 49 teeth collected by RERF, of which 4 were from survivors with unknown doses. Six had DS02 doses and one of them was a factory worker. As the total-number of the cases was small, we could not evaluate any trend of dose bias. We proposed that Nagasaki University collect more tooth samples of A-bomb survivors as a part of their Global Center of Excellence program and they agreed to do so.

RP 1-92 Radiation dose estimates using tooth samples. Part 2. Use of electron spin resonance on tooth enamel from Hiroshima atomic-bomb survivors

Hirai Y (G), Nakamura N (CS), Kodama Y (G), Wada T, Rühm W, Wallner A

The purpose of the study is to estimate individual doses using tooth enamel by the ESR technique and to compare the results with DS02 doses, and with chromosome aberration frequencies in lymphocytes, from the same donors.

1. Two manuscripts regarding measurements of neutron exposures by means of accelerator mass spectrometry ($^{41}Ca/^{40}Ca$ ratio) were submitted to *Radiation Research* and were accepted for publication.
2. ESR-estimated doses of 90 molars were compared with the cytogenetic doses based on translocation frequency (by FISH method and/or Giemsa method) of the same survivors. The two sets of biologically-derived doses agreed closely to each other (Figure 1), other than a few exceptions which may appear to be mostly explicable from other information about those cases. Because the biodosimetric data by the two completely different methods (ESR and FISH and/or Giemsa) agreed well with each other, it appears that the results validated the two methods, which may allow us to use chromosome data which had already been obtained from 4,000 A-bomb survivors. We may also be able to evaluate the possible extent and direction of dose bias in the mean DS02 doses. We are collaborating with the Department of Statistics in a proposal to use the ESR and chromosome aberration data as "instrumental variables" to provide adjusted estimates of dose; this proposal is currently under review.
3. When ESR doses or cytogenetic doses were compared with DS02 gamma-ray doses, the individual points showed a wider distribution (Figure 2). The results suggest that there may exist dose-assignment problems among people whose estimated doses are around 1 Gy.

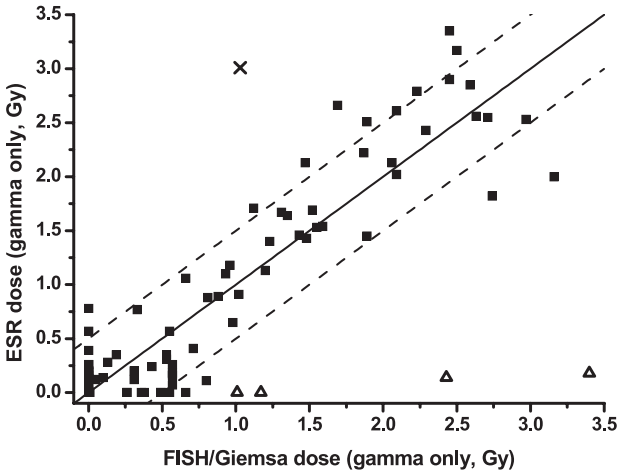


Figure 1. ESR-estimated ^{60}Co gamma-ray equivalent dose in tooth enamel in relation to cytogenetically-derived lymphocyte dose from translocation frequency assuming that the radiosensitivity is the same between bone marrow stem cells and blood lymphocytes. The solid line indicates $y = x$, and the dotted lines indicate $y = x \pm 0.5$ (not regression lines).

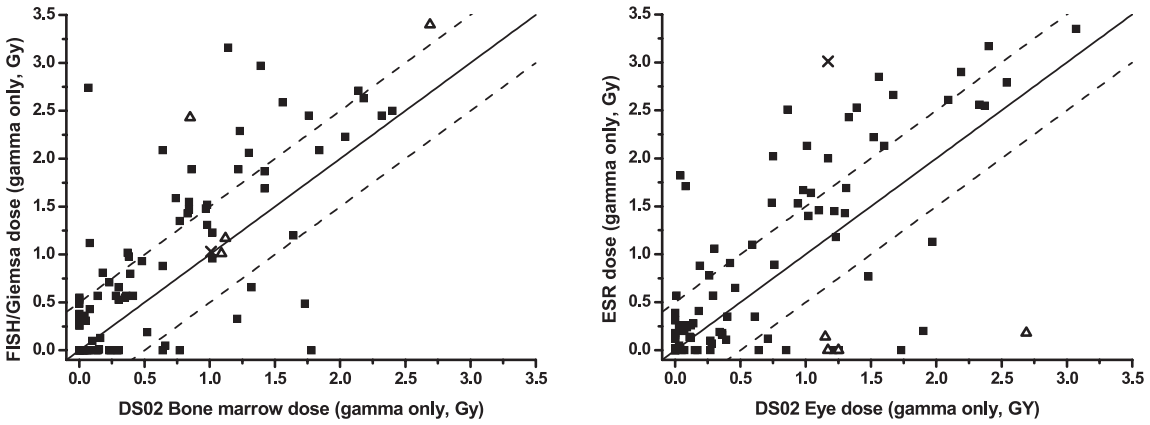


Figure 2. Cytogenetic dose (gamma-ray dose; left panel) in blood lymphocytes or ESR-estimated dose (right panel) in tooth enamel in relation to DS02 dose (bone marrow or eye dose). The solid lines indicate $y = x$, and the dotted lines indicate $y = x \pm 0.5$ (not regression lines).

RP 10-86 Radiation dose estimates using tooth samples. Part 1. Collection of tooth samples from A-bomb-exposed people in Hiroshima and Nagasaki

Hirai Y (G), Nakamura N (CS), Fujiwara S (CH), Akahoshi M (CN)

The collection of teeth from A-bomb survivors is the first part of the project to estimate the radiation doses of A-bomb survivors using the ESR method. We started collecting teeth from Hiroshima AHS participants in April 1987, and in November 2004 from Nagasaki AHS participants. As of March 2010, we have collected 1,482 tooth samples from Hiroshima AHS participants and 49 from Nagasaki AHS participants (Table). Nearly 20% of the collected samples are suitable for ESR measurement.

Table. Cumulative number of teeth

DS02 kerma dose (total cGy)	Total cases collected	
	Hiroshima	Nagasaki
0	294 (1)*	15 (3)
1–30	364 (5)	0 (0)
31–100	313 (0)	5 (0)
101 and over	267 (5)	13 (1)
Not available	244 (1)	16 (3)
	Total 1,482 (12)	Total 49 (7)

* Numbers in parentheses represent cases collected during April 2009–March 2010.

RP 18-59 Shielding survey and dosimetry study

Cullings HM (S), Grant EJ (EH), Watanabe T (EH), Funamoto S (S), Sakata R (EH)

Since well-characterized survivor dose estimates are essential to RERF research, the purpose of this protocol is to refine estimates of tissue kerma and organspecific absorbed doses for Hiroshima and Nagasaki atomic-bomb survivors and to characterize the uncertainties in these estimates. Workers in the Department of Epidemiology, under the aegis of the RERF Dosimetry Committee, completed vetting and re-entering data from original source documents on survivor locations, most notably in regard to restoring digits for ten-yard precision in map coordinates that were truncated in earlier years at ABCC, and Statistics provided an initial evaluation of resulting changes in dose estimates. Dr. Cullings worked with key members of the Dosimetry Committee in acquiring special mosaics of geometrically corrected pre-bombing aerial photographs of both cities from a contractor, which will be used for improving accuracy of survivor locations by exactly locating the neighborhood drawings of ~22,000 survivors with shielding histories. These photographic maps will also allow special "rubber sheeting" alignment of the U.S. Army maps to reduce local distortions and allow more accurate transformations of the U.S. Army map coordinates of survivors who lack shielding histories.

Regarding doses from residual radioactivity, workers in the Department of Epidemiology finished entering data from original source documents on individual survivors' early entry into the cities after the bombings and Dr. Cullings made progress in several areas including estimation of individual survivor doses from external exposure to gamma rays in 1) known areas of radioactive fallout and 2) worst-case scenarios of early entry on specific days into areas near the hypocenter affected by soil activation. He also completed a calculation of the internal radiotoxicity of activated soil near the hypocenters.

Several collaborations related to estimating and correcting for dose uncertainty continued with external investigators, including the completion of work by Dr. Pierce (Oregon, USA) on evaluating the effects of simulated dose errors on risk estimation, and a favorable NIH review of a grant application by Dr. C.Y. Wang (Fred Hutchinson Cancer Research Center, Washington State, USA) to develop functional methods for adjusting for dose errors. The Table shows a summary of doses calculated for LSS members in the Nishiyama fallout area in Nagasaki, from external exposure to gamma rays, based on early survey data, in comparison to doses received directly from the Nagasaki bomb. Nishiyama was one of two known fallout areas, one in each city, and had a much larger deposition than the fallout area in Hiroshima.

Atomic-bomb Dosimetry Studies Publications

Manuscripts in Press

⌘Hirai Y, Inoue T, Nakano M, Ohtaki K, Kodama Y, Nakamura N: Comparison of ESR-estimated dose in tooth enamel with chromosomally-estimated dose in blood lymphocytes among atomic bomb survivors. Hiroshima Igaku [Journal of the Hiroshima Medical Association] (Proceedings of the 50th Late A-bomb Effects Research

Table. Nishiyama fallout area in Nagasaki: Dose estimates based on isodose-rate contours Pace and Smith*

Location	Source	Survivors	Minimum dose, mGy	Maximum dose, mGy	Average dose, mGy	Person-gray
Fallout area	Fallout	1,754	7.7	421	101	**177** (Protracted)
Fallout area	Direct (DS02)	1,754	0	3,770	77	135
All Nagasaki survivors	Direct (DS02)	28,136				**3,970**

* ABCC TR 26-59, integrated to infinite time, not corrected for DDREF, shielding, long-term weathering, etc.

Meeting, 2009) (Japanese)
⌘Sato Y, Hoshi M, Ohtaki M, Maruyama H, Cullings HM, Kawakami H: Using geographic information system (GIS) for improvement of Hibakusha location accuracy. Hiroshima Igaku [Journal of the Hiroshima Medical Association] (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

Atomic-bomb Dosimetry Studies Oral Presentations

❖Cullings HM, Egbert SD, Wieser A, Funamoto S, Nakamura N. Estimated gamma doses to the teeth of the Japanese atomic-bomb survivors. Late Health Effects of Ionizing Radiation, 4–6 May 2009, Washington DC, USA
❖Cullings HM. Size and structure of the estimated uncertainty in radiation doses received by the atomic-bomb survivors in Hiroshima and Nagasaki. "Impact of Uncertainty in Dose to the Dose Response" Workshop, 8 May 2009, Washington DC, USA
❖Hirai Y, Inoue T, Nakano M, Ohtaki K, Kodama Y, Nakamura N. Comparison of ESR-estimated dose in tooth enamel with chromosomally-estimated dose in blood lymphocytes among atomic-bomb survivors. The 50th Late A-bomb Effects Research Meeting, 7 June 2009, Hiroshima
❖Hirai Y, Nakamura N. ESR dose estimation using tooth enamel from A-bomb survivors. The 13th ESR Forum Research Meeting, 17 July 2009, Kyoto
❖Cullings HM, Patil GP. Selection and application of spatial scan statistics for geospatial hotspotting of cancer incidence in the Japanese atomic-bomb survivors. The 53rd Annual Meeting of the Australian Mathematical Society, 28 September–1 October 2009, Adelaide, Australia
❖Hirai Y, Inoue T, Nakano M, Ohtaki K, Kodama Y, Nakamura N. ESR dose estimation using tooth enamel from Hiroshima A-bomb survivors: V. ESR gamma dose vs chromosome gamma dose. The 52nd Annual Meeting of the Japan Radiation Research Society, 11–13 November 2009, Hiroshima
❖Cullings HM, Furukawa K, Grant EJ, Joshi S, Patil GP. Application of geospatial hotspotting to cancer incidence data for the Japanese atomic-bomb survivors. International Conference on "Frontiers of Interface between Statistics and Sciences," 30 December 2009–2 January 2010, Hyderabad, India
❖Cullings HM. The spatial covariance structure of ^{137}Cs measured in soil cores collected by Hiroshima University. Workshop on Black Rain of the Hiroshima Atomic Bomb and Related Studies, 3–4 March 2010, Hiroshima