Active Research Protocols by Study Program

1 April 2010–31 March 2011

The 103 research protocols (RPs) including smaller-scale Type A protocols 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, A2-10, A1-09, A11-08, A9-08, A7-08, A3-08, A1-08

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 SM, 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.

Significantly high RRs for all causes of death were observed among current (Male, RR = 1.54, Female, RR = 1.56) and ex-smokers (Male, RR = 1.29, Female, RR = 1.35). Results by 10-year birth categories showed a tendency for later birth cohorts of current and ex-smokers to have higher RRs. Increased RRs with decreasing age started smoking were observed. This increase of RR was pronounced among subjects who were born after 1920 compared with those born before 1920. Significant RRs among current smokers were observed for almost all cause-specific deaths in both sexes, but particularly for cancer of the lung, mouth, pharynx, larynx, and esophagus, and chronic obstructive pulmonary disease (COPD). Dose-responses with smoking intensity were observed among males for those causes plus ischemic heart disease. The RR of current smokers for lung cancer mortality among males (RR = 6.6, 95% CI; 4.45–8.96) was comparable in magnitude with those obtained in previous studies in Japan. RRs for lung cancer, COPD, and other diseases were higher among subjects who were born after 1920 than those born before 1920.

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 were recruited as part of the mail survey; this represents a major collaboration between the Departments of Epidemiology and Clinical Studies. A questionnaire was sent to 5,202 LSS subjects who were under 10 years of age at the time of bombings and eligible for recruitment into the Adult Health Study, and has been responded to by 3,322 individuals, about 2,000 of whom have expressed their intention to participate in the health examinations.

Next, a pilot study was conducted as part of the mail survey to test the feasibility of collecting saliva samples for extracting subjects' DNA. Questionnaires both with and without request for saliva sample donation were each mailed to 500 LSS subjects. The sample collection rate among the subjects receiving the request was low, at 16%. Thus, a decision was made to conduct the mail survey for the remaining subjects without the request for saliva sample donation.

Mailing of the questionnaire was completed by the end of March for a total of about 24,000 LSS subjects, including those requested to participate in the AHS and the subjects of the saliva sample collection feasibility study. The questionnaire was responded to by 75% of the questionnaire recipients (excluding cases in which questionnaire was returned as undeliverable and individuals who were found to be deceased by reports from families or other means).

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

Li CI, Nishi N, 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 published by an international journal (*Cancer Research* 2010; 70(18):7187–98), and this study terminated.

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.

A manuscript on analyses of updated cancer and non-cancer mortality data (through 2003) using the DS02 has been submitted to a journal. The risk of total solid cancers from radiation increases almost linearly over the full-dose range.

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. Presently, detailed analyses of respiratory and digestive diseases are underway. There was no evidence of a radiation effect on infectious or external causes of death.

A significant linear dose response, with an assumed threshold of about 0.4 Gy, was projected for risk of early natural menopause among female A-bomb survivors (Figure 1), suggesting that exposure to 1 Gy radiation has resulted in about 34 excess female survivors who have experienced

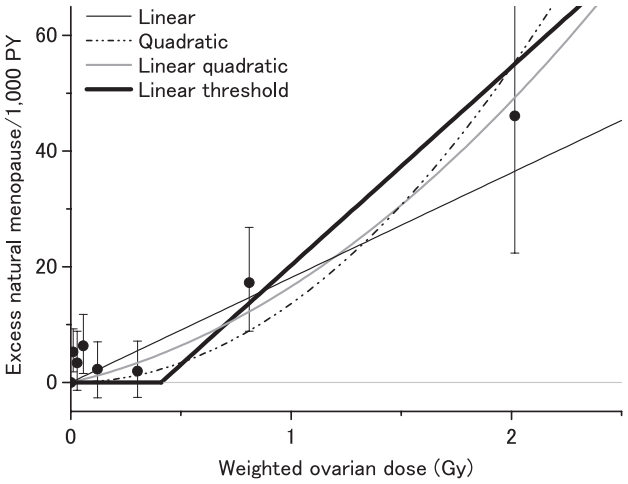

Figure 1. Dose-response relationship of radiation and early natural menopause in female survivors. Linear model with a threshold of 0.4 Gy was best fit. Closed circles are point estimates of excess relative risk for specific dose categories and whiskers are 95% confidence intervals.

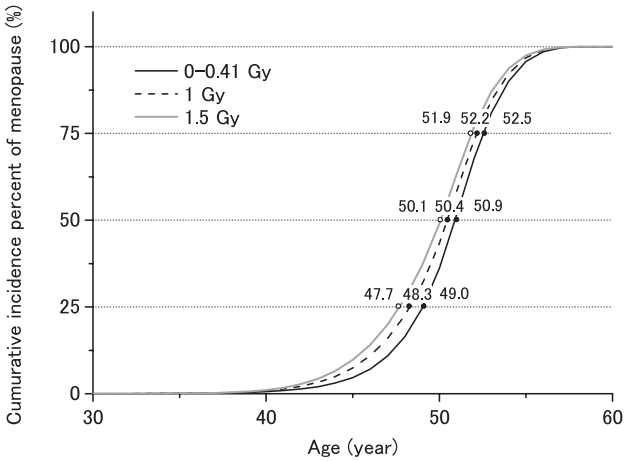

Figure 2. Cumulative incidence curves of menopause in female survivors. The median age at which they experienced natural menopause was earlier by 0.3 year in those exposed to radiation at 1.0 Gy than in non-exposed.

natural menopause at age 50 per 1,000 person-years. This is equivalent to a radiation-associated decrease in median age of natural menopause by 0.3 years (Figure 2).

Generally, breast cancer risk shows association with female reproductive history. However, no significant difference was observed in the relationship between time of radiation exposure relative to reproductive history (i.e., before menarche, between menarche and the first childbirth, and after the first childbirth) and breast cancer risk, and a significant increase by about 100–150% following exposure to 1 Gy was observed for all categories (RP A9-08).

At the end of 2006, 39% of LSS subjects, including 85% 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, 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 was conducted on radiation risks for cancer and non-cancer deaths and their temporal patterns. The analyses indicated that the sex-averaged ERR of solid cancer mortality five or more years after birth at attained age 50 yrs among *in utero* survivors (n = 2,467) was 0.83 (95% confidence interval [CI]: 0.00, 2.83), which was lower than that among those exposed in young childhood (less than 6 years of age at time of

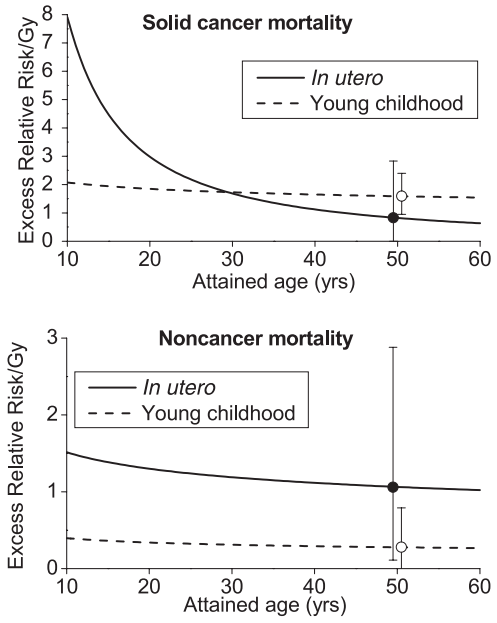

Figure. Comparison of cancer and non-cancer mortality risks between *in utero* and childhood exposure groups (1950–2003)

bombings, n = 15,461), but the difference was not statistically significant (p = 0.31). The temporal patterns between the two groups differ significantly (p < 0.04). 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 higher in the *in utero* exposure group (1.06; 95% CI: 0.11, 2.88) than in the childhood exposure group (0.28; 95% CI: –0.03, 0.79); however, the difference was not statistically significant (p = 0.24). The temporal patterns for the *in utero* and childhood exposure groups were similar (Figure).

Although the analyses suggested that solid cancer mortality was lower among the *in utero* exposure group than in the childhood exposure group, as the potential number of excess cancer cases will increase appreciably in the next few decades, it is important to continue the follow-up using both mortality and cancer incidence as endpoints.

RP-A2-10 A semi-parametric survival extrapolation method: Model validation using RERF cohort

Fang CT, Wang JD, Hwang JS, Hsu WL (S), Furukawa K (S), Kasagi F (EH), Soda M (EN), Suyama A (EN) , Ozasa K (EH), Cullings HM (S)

The knowledge that how long patients can expect to live after the diagnosis of a disease is essential for cost-effectiveness evaluation of medical interventions. Our research group has developed a semi-parametric survival extrapolation method based on logit survival ratio between patient cohort and a reference population. Our method is built on one assumption that the excess hazard associated with a particular type of disease maintains constant over time. In mathematical terms, this means that the logit survival ratio curve will converge to a straight line over time and therefore allows linear extrapolation. The method has been proven to be accurate for short-term projection of Taiwanese HIV-infected patients. However, due to limited follow-up time, we had not yet been able to validate its accuracy in life-long projection. The Life Span Study (LSS) cohort of the atomic-bomb survivors from Hiroshima and Nagasaki is unique that maintains one of the longest follow-up data in the world and will allow us to empirically examine the robustness of the constant excess hazard assumption in life-long projection scenario. We propose to examine whether the logit survival ratio curve between atomic-bomb survivors with (1) with or without radiation exposure or (2) a specific cancer (e.g., leukemia, stomach cancer, lung cancer, liver cancer, colon cancer, breast cancer, and pancreatic cancer) and Japanese reference population will converge to a straight line over time. In addition, we would test different strategies of estimating the slope of the logit of survival ratio.

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 other western 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.

Results indicated a significant quadratic dose relationship between radiation dose and possible chronic renal disease mortality, which was similar in shape to that observed between radiation and incidence of hypertension in this population. Our results suggest that renal dysfunction could be part of the mechanism causing increased CVD risk after whole body irradiation, a hypothesis that deserves further study.

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. Dr. Adams' manuscript is currently in RERF internal review and will be submitted to *Radiation Research* in 2011.

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. 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.

The project employed 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 were age at menarche, age at first birth, and radiation dose. Variables that were of use in assessing confounding include body mass index, cancer type, height, hormone receptor status, and parity. The primary outcome of interest was 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 involved 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 (Figure). A manuscript was published (McDougall et al., *Cancer Epidemiology, Biomarkers and Prevention* 2010; 19[7]:1746–54), and this RP was terminated.

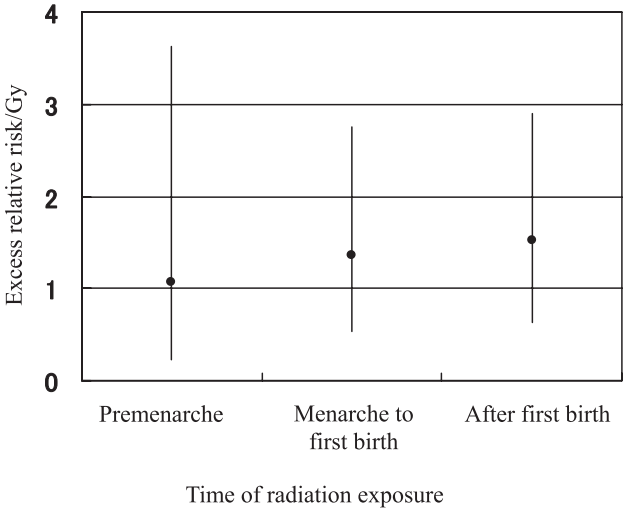

Figure. Risk of radiation for breast cancer by female reproductive history.

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.

The first of three manuscripts from this project is in RERF internal review. That manuscript used a full cohort design and included lifestyle factors where available. Lifestyle factors included smoking, drinking, vegetable consumption, fruit consumption, and highest attained educational level. Results indicated that radiation risk estimates were not modified by lifestyle factors despite the strong dependence of UC on smoking.

The last lifestyle factor under consideration is occupational exposures to classes of chemicals that cause UC. Using a stratified case-cohort design, occupational exposures to aromatic amines and polycyclic hydrocarbons were determined via recoding survey data and using a job exposure matrix. Two manuscripts are in preparation. The first is a methodology paper on the occupational exposure assignments. The second manuscript reports the results of the stratified analysis.

Results for the final manuscript will be presented at the IEA World Congress of Epidemiology (Edinburgh, Scotland) in August 2011. This project will be successfully terminated in FY2011.

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) were utilized.

Results indicate that increasing BMI was significantly associated with increasing risks of colon cancer, however, radiation risk estimates were not modified by those associations.

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 has submitted a manuscript to RERF's internal review. The project will be successfully terminated in FY 2011.

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 the previous 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. Dr. Furukawa recently completed a manuscript on using multiple imputation of missing data on smoking for risk estimation of lung cancer, which is an example of an alternative to full-likelihood Bayesian MCMC calculations that is at present more computationally feasible.

Life Span Study Publications

RERF Reports (RR)

♦ Li CI, Nishi N, McDougall JA, Semmens EO, Sugiyama H, Soda M, Sakata R, Hayashi M, Kasagi F, Suyama A, Mabuchi K, Davis S, Kodama K, Kopecky KJ: Relationship between radiation exposure and risk of second primary cancers among atomic bomb survivors. Cancer Research 2010 (September); 70(18):7187–98. (RR 1-10)

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♦ McDougall JA, Sakata R, Sugiyama H, Grant EJ, Davis S, Nishi N, Soda M, Shimizu Y, Tatsukawa Y, Kasagi F, Suyama A, Ross P, Kopecky KJ, Li CI: Timing of menarche and first birth in relation to risk of breast cancer in A-bomb survivors. Cancer Epidemiology, Biomarkers and Prevention 2010 (July); 19(7):1746–54. (RR 2-10)

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Other Journal Publications

♦ Grant EJ, Ozasa K: Ionizing radiation. Tuncer AM, ed. Asian Pacific Organization for Cancer Prevention Cancer Report 2010. Ankara, Turkey: New Hope in Health Foundation; 2010 (April), pp 49–52.

♦ Neriishi K: A hypothesis: Effects of malnutrition exposure in prenatally or natally exposed A-bomb survivors. Igaku no Ayumi [Journal of Clinical and Experimental Medicine] 2010 (November); 235(8):863–6. (Japanese) (related to *Adult Health Study*)

♦ Shore RE: Implications of radiation epidemiologic data for risk assessment and radiation protection. Health Physics 2011 (March); 100(3):306–8. (Abstracts of the Biological Consequences and Health Risks of Low-level Exposure to Ionizing Radiation, Richland, Washington, USA, 3–5 May 2010)

♦ Suyama A: A-bomb radiation effects on human body. Nagasaki Association for Hibakusha's Medical Care (NASHIM), ed. Hibakusha in the 21st Century—Hibakusha in the World and Forefront of Study on Radiation Diseases. Nagasaki: Nagasaki Press; 2011 (March), pp 118–28. (Japanese) (related to *Adult Health Study*)

Manuscripts in Press

⌘ Ozasa K, Shimizu Y, Sakata R, Sugiyama H, Grant EJ, Soda M, Kasagi F, Suyama A: Risk of cancer and non-cancer diseases in the atomic bomb survivors. Radiation Protection Dosimetry.

⌘ Samartzis D, Nishi N, Hayashi M, Cologne JB, Cullings HM, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F: Exposure to ionizing radiation and development of bone sarcoma: New insights based on atomic-bomb survivors of Hiroshima and Nagasaki. Journal of Bone and Joint Surgery. American Volume. (related to *Tumor and Tissue Registries*)

Life Span Study Oral Presentations

❖ Shore RE. Assessing the health effects of low-dose radiation exposure: Data from the Japanese atomic bomb survivors. EPICOH-MEDICHEM 2010, 21–24 April 2010, Taipei, Taiwan

❖ Shore RE. Implications of radiation epidemiologic data for risk assessment and radiation protection. Biological Consequences and Health Risks of Low-level Exposure to Ionizing Radiation, 3–5 May 2010, Richland, Washington, USA

❖ Ozasa K. Risk of cancer and non-cancer diseases in the atomic-bomb survivors. The 3rd Asian and Oceanic Congress on Radiation Protection, 24–28 May 2010, Tokyo

❖ Ozasa K, Shimizu Y, Kasagi F, Grant EJ, Sakata R, Sugiyama H, Soda M, Suyama A. Radiation risk of major causes of death in the atomic-bomb survivors, 1950–2003.

2010 American Statistical Association's Conference on Radiation and Health, 13–16 June 2010, Annapolis, Maryland, USA

❖ Ozasa K, Shimizu Y, Kasagi F, Grant EJ, Sakata R, Sugiyama H, Soda M, Suyama A. Radiation risk of cancer mortality in the atomic-bomb survivors, 1950–2003. Scientific Meeting for Cancer Prevention 2010 Sapporo, 15–16 July 2010, Sapporo

❖ Grant EJ, Sakata R, Shimizu Y, Kasagi F, Sugiyama H, Soda M, Suyama A, Ozasa K. The effect of lifestyle factors on urothelial carcinoma radiation risk estimates among atomic bomb survivors. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA

❖ Sakata R, Shimizu Y, Hsu WL, Hayashi M, Soda M, Suyama A, Ozasa K. Determination of relationship between radiation dose and age at menopause on female atomic bomb survivors. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA

❖ Shore RE, Ozasa K, Kasagi F, Imaizumi M, Tatsukawa Y. Are those exposed *in utero* the most radiosensitive population?—The Japanese A-bomb experience. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA (related to *Adult Health Study* and *Special Clinical Studies*)

❖ Kasagi F, Sugiyama H, Sakata R, Suyama A, Ozasa K. Solid cancer mortatily risk in the *in utero* cohort exposed to the atomic bomb radiation. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Sakata R, Shimizu Y, Hsu WL, Hayashi M, Soda M, Suyama A, Ozasa K. Early onset of menopause among female atomic bomb survivors. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Kodama K, Shimizu Y, Douple EB, Shore RE. What and how important are the late non-cancer risks from radiation therapy?—With special reference to cardiovascular disease. The 52nd Annual Meeting of the American Society of Therapeutic Radiation Oncologists, 31 October–4 November 2010, San Diego, California, USA (related to *Adult Health Study*)

❖ Shore RE, Ozasa K, Shimizu Y, Sakata R, Furukawa K, Preston DL, Hsu WL, Kodama K. What light do the cancer studies of A-bomb survivors shed on late effects from radiation therapy? The 52nd Annual Meeting of the American Society of Therapeutic Radiation Oncologists, 31 October–4 November 2010, San Diego, California, USA

❖ Kasagi F. Health effects from radiation among A-bomb survivors. Lectures on Radiation Epidemiology Research for 2010, 9 November 2010, Tokyo

❖ Shore RE, Ozasa K, Shimizu Y, Douple EB, Kodama K. The Japanese A-bomb survivor data and studies of low-dose effects. The 4th International Conference: "Chronic Radiation Exposure: Low-dose Effects," 9–11 November 2010, Chelyabinsk, Russia

❖ Shore RE, Ozasa K, Shimizu Y, Kasagi F, Furukawa K, Hsu WL, Preston DL, Neriishi K, Suyama A, Kodama K. Japanese atomic bomb survivor data and studies of low-dose effects. The 9th Annual Gilbert W. Beebe Symposium, 1 December 2010, Washington DC, USA

❖ Kodama K. Radiation exposure and cardiovascular disease risk and its potential pathways: Hiroshima and Nagasaki atomic bomb survivors data. The Exploratory Workshop on Radiation-induced Vascular Effects Resulting from Low Dose, 7–10 December 2010, Bombon, France (related to *Adult Health Study*)

❖ Ozasa K, Shimizu Y, Kasagi F, Grant EJ, Sakata R, Sugiyama H, Pham TM, Soda M, Suyama A. Risk of radiation for cancer and non-cancer diseases. The 21st Annual Scientific Meeting of the Japan Epidemiological Association, 21–22 January 2011, Sapporo

❖ Sakata R, Shimizu Y, Hayashi M, Soda M, Suyama A, Ozasa K. Consistency in mail survey responses on age at menopause and factors affecting the age at menopause. The 21st Annual Scientific Meeting of the Japan Epidemiological Association, 21–22 January 2011, Sapporo

❖ Shore RE. Cardiovascular risk after low-dose radiation exposure: A timely question. Workshop: Planning Pilot Study of One Million U.S. Radiation Workers, 3 February 2011, Oak Ridge, Tennessee, USA (related to *Special Clinical Studies*)

❖ Shore RE, Shimizu Y, Yamada M, Kodama K. Cardiovascular disease after A-bomb radiation exposure. The 1st International Symposium of Research Institute for Radiation Biology and Medicine, Hiroshima University, 3–4 March 2011, Hiroshima (related to *Adult Health Study*)

Research Protocols 2-11, 7-10, 7-09, 3-07 (Platform Protocol), 2-75 (Platform Protocol), A3-09
Adult Health Study (AHS)

RP 2-11 Study of arteriosclerosis in the Adult Health Study population (Part 2. Analysis of the cytokine network regulating differentiation of mesenchymal stem cells in artery)

Takahashi I (CH), Ohishi W (CH), Hayashi T (R), Cologne JB (S), Takahashi T, Kusunoki Y (R), Ozasa K (EH), Kihara Y, Matsumoto M, Fujiwara S (CH)

Reports regarding therapeutic irradiation of the human head and animal experiments, as well as reports involving mortality and incidence of arteriosclerotic diseases among A-bomb survivors, have suggested that high-dose radiation induces arteriosclerosis, but the mechanism of such induction is unclear. It is difficult to explain the entire picture of the complex clinical condition of arteriosclerosis with the conventional hypothesis that arteriosclerosis is an inflammatory disease. Tissue damage is probably of primary importance in relatively high-dose-radiation-induced arteriosclerotic changes. In this study, therefore, we will consider arteriosclerosis based on the "inflammation-response-to-injury" hypothesis. We hypothesize that diseases related to "artery-bone metabolism-immunity" are abnormalities in differentiation and proliferation of arterial mesenchymal tissue. Thus, we also will measure several multi-functional cytokines in 2,100 Adult Health Study (AHS) subjects (including those exposed at young ages). That cross-sectional study is designed to test our hypothesis that "abnormalities in the cytokine network initiated by tissue damage at the time of radiation exposure induce abnormalities in mesenchymal tissues." We will obtain measurements of arteriosclerotic markers (augmentation index [AI]; brachial-ankle pulse wave velocity [baPWV]; ankle-brachial index [ABI]; intima-media thickness [IMT]; calcification of aortic arch and/or abdominal aorta) and cytokines (pentraxin [PTX]-3; osteopontin [OPN]; osteoprotegerin [OPG]; receptor activator of nuclear factor [NF]-κB ligand [RANKL]; vascular endothelial growth factor [VEGF]-A; high mobility group box [HMGB]-1; apolipoprotein [Apo]-J, also called clusterin; interleukin [IL]-17), which will be measured once during the four years (two cycles) starting in 2010 that constitute our study period. We also will measure reactive oxygen species (ROS), which act as proliferation signals for mesenchymal stem cells. We will then examine if the "cytokine network" functions to either moderate or mediate the radiation effect upon atherosclerotic cardiovascular outcomes.

RP 7-10 Study of body composition of the Hiroshima Adult Health Study population

Tatsukawa Y (CH), Fujiwara S (CH), Harris TB, Misumi M (S), Ohishi W (CH), Masunari N (CH), Yamada M (CH), Oyama H, Kasagi F (EH)

Background: Some recent results from studies of atomic-bomb (A-bomb) survivors have shown a positive association between radiation dose and incidence of arteriosclerotic diseases such as hypertension and myocardial infarction (MI). The underlying mechanisms of radiation dose effects on arteriosclerotic diseases, however, remain elusive. In addition, there are limited numbers of reports on the presence or absence of racial differences in the health effects of body composition.

Objectives: The objectives of this prospective study include: 1) testing whether radiation exposure is related to increased incidence of arteriosclerotic diseases and their risk factors through modifications in body composition; 2) examining effects of body composition modifications, particularly aging-related loss of muscle mass (sarcopenia), on the health of the Japanese people, such as the prevalence and incidence of arteriosclerotic diseases, and associated risk factors and mortality; and 3) comparing Hiroshima Adult Health Study (AHS) participants and U.S. Health, Aging, and Body Composition (ABC) Study participants, with respect to potential racial differences in health effects associated with body composition (international collaborative study).

Methods: Study subjects will total approximately 2,200 Hiroshima AHS participants who underwent whole-body composition examination by dual energy X-ray absorptiometry (DEXA) during the period 1994–1996. In the Hiroshima AHS, measurements of whole-body/regional (trunk, limb, etc.) fat mass (FM), lean mass (LM), and bone mineral content (BMC) were conducted by DEXA starting in 1994. Those data are already stored in a database and available for use. In this prospective study, we will examine relationships between DEXA-based body composition and radiation dose, and the prevalence and incidence of arteriosclerotic diseases plus associated risk factors and mortality. Mortality endpoints will include all causes, ischemic heart disease, stroke, and possibly other cardiovascular diseases, if feasible.

This study may be useful to elucidate mechanisms of the relationship of radiation dose to arteriosclerotic diseases and their risk factors. Furthermore, the international collaborative study should be meaningful for both the A-bomb survivors and the general Japanese population.

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 started 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, 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.

As of November 2010, we have examined 1,949 subjects in Hiroshima and Nagasaki who were 0–9 years old at the time of atomic bombings. Although the participation rate was slightly lower than expected, 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)

Objectives: 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.

Background: 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. We have added about 1,900 young exposed subjects (<10 years old at the bombings) to the study in 2008–2010.

Study methods: The study attempts to examine differences in diseases or pre-clinical disorders by radiation exposures. During the 25th cycle (July 2006–June 2008), 3,609 individuals were examined, representing approximately 70% of the AHS cohort still living in the contact areas of interest.

Study progress: Health examinations have been continued. The biological specimens collected are used for clinical determinations and stored for future studies. Evaluation of possible interactions between radiation and infectious agents or hormones and cancer risk, and phenotypic and genetic factors have been conducted using stored specimens. Tissue collection of operated cataract tissue was begun in 2009. Other recently initiated radiation-related studies include ones on: liver stiffness, chronic kidney dysfunction/disease, cardiovascular disease, and pre-clinical measurements of atherosclerosis.

Results: Reports of new findings include: metabolic cardiovascular risk factors and subclinical hypothyroidism, longitudinal trends of total white blood cell, biomarkers of radiosensitivity in A-bomb pregnant survivors, lifetime risk of stroke, and others.

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

In November 2009, we started data collection to analyze association of prevalent CKD cases with radiation and CKD risk factors.

Adult Health Study Publications

RERF Reports (RR)

♦ 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 2010 (July); 51(4):431–9. (RR 5-09) (related to *Special Clinical Studies*)

[Abstract] In studying the late health effects of atomic-bomb (A-bomb) survivors, earlier findings were that white blood cell (WBC) count increased with radiation dose in cross-sectional studies. However, a persistent effect of radiation on WBC count and other risk factors has yet to be confirmed. The objectives of the present study were 1) to examine the longitudinal relationship between A-bomb radiation dose and WBC and differential WBC counts among A-bomb survivors and 2) to investigate the potential confounding risk factors (such as age at exposure and smoking status) as well as modification of the radiation dose-response. A total of 7,562 A-bomb survivors in Hiroshima and Nagasaki were included in this study from 1964–2004. A linear mixed model was applied using the repeated WBC measurements. During the study period, a secular downward trend of WBC count was observed. Radiation exposure was a significant risk factor for elevated WBC and differential WBC counts over time. A significant increase of WBC counts among survivors with high radiation dose (>2 Gy) was detected in men exposed below the age of 20 and in women regardless of age at exposure. Effects on WBC of low dose radiation remain unclear, however. Cigarette smoking produced the most pronounced effect on WBC counts and its impact was much larger than that of radiation exposure.

♦ Shore RE, Neriishi K, Nakashima E: Epidemiological studies of cataract risk at low to moderate radiation doses: (Not) seeing is believing. Radiation Research 2010 (December); 174(6):889–94. (related to *Special Clinical Studies*)

[Abstract] The prevailing belief for some decades has been that human radiation-related cataract occurs only after relatively high doses; for instance, the ICRP estimates that brief exposures of at least 0.5–2 Sv are required to cause detectable lens opacities and 5 Sv for vision-impairing cataracts. For protracted exposures, the ICRP estimates the corresponding dose thresholds as 5 Sv and 8 Sv, respectively. However, several studies, especially in the last decade, indicate that radiation-associated opacities occur at much lower doses. Several studies suggest that medical or environmental radiation exposure to the lens confers risk of opacities at doses well under 1 Sv. Among Japanese A-bomb survivors, risks for cataracts necessitating lens surgery were seen at doses under 1 Gy. The confidence interval on the A-bomb dose threshold for cataract surgery prevalence indicated that the data are compatible with a dose threshold ranging from none up to only 0.8 Gy, similar to the dose threshold for minor opacities seen among Chernobyl clean-up workers with primarily protracted exposures. Findings from various studies indicate that radiation risk estimates are probably not due to confounding by other cataract risk factors and that risk is seen after both childhood and adult exposures. The recent data are instigating reassessments of guidelines by various radiation protection bodies regarding permissible levels of radiation to the eye. Among the future epidemiological research directions, the most important research need is for adequate studies of vision-impairing cataract after protracted radiation exposure.

Other Journal Publications

♦ Fujiwara S: Path traveled by the Radiation Effects Research Foundation's research. Hiroshima Igaku [Journal of the Hiroshima Medical Association] 2010 (April); 63(4):244–7. (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

♦ Miles EF, Tatsukawa Y, Funamoto S, Kamada N, Nakashima E, Kodama Y, Seed TM, Kusunoki Y, Nakachi K, Fujiwara S, Akahoshi M, Neriishi K: Biomarkers of radiosensitivity in A-bomb survivors pregnant at the time of bombings in Hiroshima and Nagasaki. ISRN Obstetrics and Gynecology 2010 (September); Volume 2011(Article ID 264978):1–11.

♦ Neriishi K: A hypothesis: Effects of malnutrition exposure in prenatally or natally exposed A-bomb survivors. Igaku no Ayumi [Journal of Clinical and Experimental Medicine] 2010 (November); 235(8):863–6. (Japanese) (related to *Life Span Study*)

♦ Suyama A: A-bomb radiation effects on human body. Nagasaki Association for Hibakusha's Medical Care (NASHIM), ed. Hibakusha in the 21st Century—Hibakusha in the World and Forefront of Study on Radiation Diseases. Nagasaki: Nagasaki Press; 2011 (March), pp 118–28. (Japanese) (related to *Life Span Study*)

♦ Global Blood Pressure Genetics Consortium (RERF: Takahashi N): Common variants in the ATP2B1 gene are associated with susceptibility to hypertension. The Japanese millennium genome project. Hypertension 2010 (November); 56(5):973–80. (related to *Biochemical Genetics Studies*)

Manuscript in Press

⌘ Takahashi I, Geyer SM, Nishi N, Ohshita T, Takahashi T, Akahoshi M, Fujiwara S, Kodama K, Matsumoto M: Lifetime risk of stroke and impact of hypertension: Estimates from the Adult Health Study in Hiroshima and Nagasaki. Hypertension Research.

Adult Health Study Oral Presentations

❖ Akahoshi M. Current status of A-bomb survivor health examinations. The 51st Late A-bomb Effects Research Meeting, 6 June 2010, Nagasaki

❖ Nakashima E, Fujii Y, Neriishi K, Minamoto A. Assessment of misclassification of binary response: Recovering information on clinically significant cataract prevalence from cataract surgery data in atomic-bomb survivors. FY2010 Joint Statistics Meeting, 5–8 September 2010, Tokyo

❖ Shore RE, Ozasa K, Kasagi F, Imaizumi M, Tatsukawa Y. Are those exposed *in utero* the most radiosensitive population?—The Japanese A-bomb experience. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA (related to *Life Span Study* and *Special Clinical Studies*)

❖ Kodama K, Ozasa K, Sugiyama H, Soda M, Suyama A, Katayama H, Shore RE. Radiation and cancer incidence in atomic bomb survivors—Effective use of cancer registry data. The 32nd Annual Meeting of the International Association of Cancer Registries, 12–14 October 2010, Yokohama (related to *Tumor and Tissue Registries*)

❖ Kodama K, Shimizu Y, Douple EB, Shore RE. What and how important are the late non-cancer risks from radiation therapy?—With special reference to cardiovascular disease. The 52nd Annual Meeting of the American Society of Therapeutic Radiation Oncologists, 31 October–4 November 2010, San Diego, California, USA (related to *Life Span Study*)

❖ Kodama K. Radiation exposure and cardiovascular disease risk and its potential pathways: Hiroshima and Nagasaki atomic bomb survivors data. The Exploratory Workshop on Radiation-induced Vascular Effects Resulting from Low Dose, 7–10 December 2010, Bombon, France (related to *Life Span Study*)

❖ Akahoshi M. A-bomb exposure and ischemic heart disease. Research Meeting of Physicians in Medical Institutions for Treatment of A-bomb Survivors, 4 February 2011, Nagasaki

❖ Akahoshi M. A-bomb exposure and ischemic heart disease. The 13th Coordination and Planning Meeting of the WHO REMPAN Collaborating Centres and Liaison Institutions, 16–18 February 2011, Nagasaki

❖ Kodama K, Ozasa K, Sugiyama H, Soda M, Suyama A, Katayama H, Shore RE, Okubo T. Radiation effects on cancer risks in the Life Span Study cohort. The 13th Coordination and Planning Meeting of the WHO REMPAN Collaborating Centres and Liaison Institutions, 16–18 February 2011, Nagasaki (related to *Tumor and Tissue Registries*)

❖ Shore RE, Shimizu Y, Yamada M, Kodama K. Cardiovascular disease after A-bomb radiation exposure. The 1st International Symposium of Research Institute for Radiation Biology and Medicine, Hiroshima University, 3–4 March 2011, Hiroshima (related to *Life Span Study*)

Research Protocols 4-10 (Platform Protocol), 1-02 (Platform Protocol)
F_1 Clinical Study

RP 4-10 Longitudinal clinical study of the F_1 offspring of A-bomb survivors

Ohishi W (CH), Fujiwara S (CH), Akahoshi M (CN), Suyama A (EN), Kasagi F (EH), Furukawa K (S), Hsu WL (S), Takahashi N (G), Satoh Y (G), Kusunoki Y (R), Yamada M (CH), Neriishi K (CH), Tatsukawa Y (CH), Takahashi I (CH), Hida A (CN), Imaizumi M (CN), Sera N (CN), Grant EJ (EH), Ozasa K (EH), Cologne JB (S), Cullings HM (S), Kodama Y (G), Katayama H (IT), Watanabe T (EH), Nakamura N (CS)

Rationale: The heritable effects of exposure to ionizing radiation have long been a public health concern. However, there are no human data on the potential risk of adult-onset multifactorial diseases in the offspring of exposed persons. This longitudinal clinical study of the F_1 offspring of A-bomb survivors will provide the first information on this important issue.

Objectives: The objectives of the Longitudinal Clinical Study of the F_1 Offspring of A-bomb Survivors (F_1 Clinical Study) are (1) to elucidate the effects of parental exposure to A-bomb radiation on the development of multifactorial diseases and subclinical conditions among the F_1 offspring of A-bomb survivors, (2) to increase the precision and reliability of risk assessments, (3) to preserve biological samples for future research studies, and (4) to contribute to the health and welfare of the F_1 population via health examinations and health guidance.

Methods: This prospective study will conduct periodic health examinations of a fixed cohort of F_1 offspring born to A-bomb survivors between 1946 and 1984. In the initial F_1 health effects study, 14,175 people indicated they were potentially willing to undergo health examinations among the 16,789 subjects of mail surveys who responded to questionnaires concerning health and living habit by mail or telephone between May 2000 and November 2008. Of those, 11,984 actually attended the Radiation Effects Research Foundation (RERF) clinic for an examination by the end of November 2008. After excluding 397 of the 14,175 because of subsequent death or contact refusal and 1,320 with currently unknown addresses, a total of 12,458 are potentially eligible.

The F_1 Clinical Study with health examinations will be carried out every four years. First, brochures on outline of the health examination will be sent to all subjects, and secondly, participation in a clinical study will be requested by telephone contact. A letter explaining the health examination and samples of informed consent forms will be sent in advance to those who express a desire to participate in the health examination. In the F_1 Clinical Study, upon obtaining informed consent from the participant, the health examination will include a sociodemographic, lifestyle and medical-history interview, physical examination, anthropometric measurements, blood pressure measurements, electrocardiogram (ECG), urinalysis, blood/biochemical tests, stool occult blood test, abdominal ultrasonography, chest X-ray examination, and so on. Multifactorial diseases such as hypertension, hyperlipidemia, diabetes mellitus,

ischemic heart disease, and stroke detected via these examinations will be analyzed in relation to radiation exposure of their parents, taking into consideration confounding factors. Blood and urine taken from those who gave their consent will be preserved for future research studies.

The F_1 Clinical Study will contribute to the health management and welfare of the F_1 offspring of A-bomb survivors by feeding back the results of the health examination, providing appropriate health guidance, and providing an outside medical referral if necessary.

The procedures and contents of the health examination, the ethical issues, data management, and so on are generally similar to the previous F_1 Clinical Study (FOCS). Future research studies using stored biological samples will be conducted after a separate detailed research protocol is prepared and approved by the usual RERF procedures.

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)

Objectives: To assess the possible genetic effects and associated long-term health consequences among children of the atomic-bomb survivors.

Background and significance: A wide variety of experimental studies have shown that radiation exposure can induce germ cell mutations and chromosome aberrations that may cause hereditary diseases. To date the studies have produced no evidence of dose-related genetic effects on the children of A-bomb survivors. But it is critically important to obtain data on less fatal but more common multi-factorial diseases and precursor conditions. The study therefore concentrated on diabetes and cardiovascular diseases or conditions.

Study methods: Prevalence study from 2002 to 2006.

Study progress: A paper on the overall prevalence of the selected multi-factorial diseases in relation to radiation dose was published. Analyses of radiation-related genetic effects on individual multi-factorial diseases such as hypertension, hypercholesterolemia, and diabetes are almost completed.

Results and conclusions: This cross-sectional study provided no evidence for an overall increased prevalence of adult-onset multi-factorial diseases in relation to parental radiation exposure among nearly 12,000 offspring of A-bomb survivors (Fujiwara et al., *Radiation Research* 2008; 170:451–7). The analyses are being conducted regarding genetic effects of parental radiation exposure for individual disease and preclinical conditions such as hypercholesterolemia, hypertension, diabetes, angina, myocardial infarction, or stroke.

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), Kyoizumi S (R), Kajimura J (R), Yoshida K (R), Hayashi T (R), Geyer SM, 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 established 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 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.

To develop an assay system for use in this study, we analyzed the time-course of IFN-γ, IL-10, IL-6, and IL-2 supernatant protein levels and also their mRNA expression levels using 16 in-house volunteers' PBMC samples after *in vitro* stimulation with flu vaccine antigen (H1N1). The levels of IFN-γ mRNA time-dependently increased up to 96 hours after *in vitro* stimulation. Although peak time varied among individuals, 96 hours seems to be appropriate as a measurement time. Supernatant IFN-γ, IL-10, and IL-6 levels in PBMCs stimulated with flu vaccine antigen time-dependently increased up to 96 hours. On the other hand, supernatant IL-2 levels increased up to 24 hours and decreased thereafter. We started a pilot vaccination effects study of 50 A-bomb survivors and 20 in-house volunteers (aged 40 or younger) during the 2010 flu vaccination season. From among 145 AHS subjects who participated in this survey, 86 people had a history of influenza vaccination within the previous five years and also planned to receive influenza vaccination from their attending physicians this coming season. Of the 86 AHS participants, 57 subjects (66%) indicated clear willingness to participate in the pilot vaccination study. The Hiroshima City Medical Association (HCMA) provided its official approval for cooperation in this study. On the basis of HCMA's cooperation, we succeeded in obtaining cooperation from 51 out of 52 doctors. Finally, we collected blood samples before and three weeks after the vaccination from 50 (88%) of the 57 subjects and the 20 in-house volunteers.

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

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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.

We have been conducting measurements in lymphocyte subsets from 1,176 of the 1,300 AHS subjects in the new AHS cohort extension. We have also analyzed intracellular ROS levels in granulocytes, monocytes, and lymphocyte subsets (naïve CD4, memory CD4, naïve CD8, and memory CD8 T cells) with blood samples from 400 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.

We have to this point investigated effects of polymorphic *NBN*, which plays crucial roles in DNA double-strand-break repair, in stomach, lung, breast, and combined solid cancers

in A-bomb survivors. SNP genotyping was performed using the TaqMan 5′ nuclease assay. Linkage disequilibrium analysis indicated that five SNP sites of the *NBN* gene region were included in one haplotype block (major haplotype alleles: *CCTCG* and *TGCGA*). In addition, two of the five SNPs are located at a putative gene regulatory region. Relative risks (RR) of cancer incidence were estimated using Cox hazard regression model: the *NBN* haplotypes were found not to be associated with cancer risk in non-exposed (<1 mGy) or low radiation dose-exposed individuals (<1 Gy). However, a particular *NBN* haplotype was significantly associated with significantly increased risk of combined solid cancers (RR: 1.38, 95% CI: 1.03–1.84) as well as increased risk of stomach cancer (RR: 2.26, 95% CI: 1.02–5.00) in individuals exposed to 1 Gy or higher. Those results suggest that the *NBN* gene polymorphisms may contribute to inter-individual differences in DNA repair capacity and consequently cancer susceptibility 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

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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 subgroup 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.

We have identified *DQA1* and *DRB1* genotypes and analyzed the relationships between particular *HLA* genotypes, risk of DM, and radiation dose in 711 DM patients (483 and 228 in Hiroshima and Nagasaki, respectively) and 1,878 controls (966 and 912 in Hiroshima and Nagasaki, respectively). Prevalence of DM increased with radiation dose (trend $p < 0.001$) in Hiroshima subjects but not in Nagasaki subjects. In particular, heavily exposed Hiroshima subjects with *DQA1*01:02-DRB1*15:01:01*, *DQA1*01:02-DRB1*16:02:01*, *DQA1*03:01-DRB1*04:05*, *or DQA1*05:01-DRB1*14:03:01* haplotype revealed significantly higher risk of DM than that of non-exposed subjects (OR = 1.73/Gy, 95%CI: 1.36 to 2.20). On the other hand, A-bomb survivors with other haplotypes did not show such elevation in prevalence of DM with radiation dose.

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.

This fiscal year, we have obtained new evidence supporting this hypothesis: each of the percentages of T_H1 and T_H2 cells in the CD4 T-cell population of A-bomb survivors significantly increased with age and radiation dose. Furthermore, the HCV-infected group revealed inverse association between time-course changes in T_H1 cell percentage and those in platelet counts, suggesting the possibility that enhanced T_H1 cell-mediated immunity may be involved in progression of viral hepatitis.

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 17,557 blood samples from 3,440 AHS participants in Hiroshima and 11,223 from 2,536 AHS participants in Nagasaki. It was determined by PCR amplification that DNA extracted from laboratory control blood stored at –80°C for 13 years on paper was not significantly affected by storage. We have also collected 2,215 blood cell samples (1,262 in Hiroshima and 631 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.

This fiscal year, intracellular ROS levels of selected lymphocyte subsets, together with the subset percentages, in 1,393 AHS subjects (Hiroshima 823 and Nagasaki 570 AHS subjects, plus Hiroshima 630 and Nagasaki 284 expanded younger AHS subjects) have been examined by flow cytometry. Measurement of intracellular ROS levels is still underway. We also measured serum inflammatory cytokines/chemokines among 449 non-AHS control samples, using the Bio-Plex Pro cytokine system. We found that cigarette smoking enhanced serum levels of IL-8, IL-12p70, and MCP-1.

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.

This fiscal year, we have cryopreserved blood cells from 2,267 AHS participants (1,388 in Hiroshima and 879 in Nagasaki) and 914 AHS participants from the newly expanded group of younger A-bomb survivors (626 in Hiroshima and 288 in Nagasaki). 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. Following a

recommendation by the Multinational Peer Review Panel, the Committee on Biological Samples requested that samples from each of the AHS participants be divided and stored both in Hiroshima and Nagasaki, with the aim of avoiding the loss of such valuable samples due to disasters and other problems. To this end, we have completed storage of at least four vials for each sample with regard to a portion of the subject population. Since samples prepared from 921 Hiroshima participants and 207 Nagasaki participants totaled fewer than four vials, however, we have embarked on the work of preparing at least four vials for each sample.

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 methods for measuring radiation-induced phosphorylated histone H2AX (γH2AX) in CD34-positive hematopoietic stem cells in peripheral blood, 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)

♦ 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-Fundamental and Molecular Mechanisms of Mutagenesis 2010 (July); 689(1–2):59–64.

 (RR 13-08)

[Abstract] Patients who received hematopoietic cell transplants have an increased risk for a new malignancy. In addition to genotoxic regimens such as radiotherapy and chemotherapy, graft-versus-host disease (GVHD) is a risk factor for development of new malignancies in long-term survivors. To understand mechanisms underlying this malignant transformation, we evaluated genomic damage in several murine models of GVHD by enumerating reticulocytes containing micronuclei (MN) in the blood after semi-allogeneic (parent-into-F1) hematopoietic cell transplantation. On day 40 after transplantation, MN frequencies were significantly increased in unirradiated (C57BL6 × DBA/2) F1 (BDF1) and (BALB/c × C57BL6) F1 (CBF1) mice that received cells from C57BL6 (B6) donors. MN frequencies were not significantly increased in F1 mice that received cells from DBA/2 or BALB/c donors. Serum levels of tumor necrosis factor-α (TNF-α) were higher after transplantation with B6 donors than with DBA/2 or BALB/c donors. The results indicate that GVHD, without irradiation, can induce genomic damage associated with inflammatory reactions manifested by increased TNF-α levels.

♦ 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 2010 (December); 174(6):870–6.

 (RR 3-09)

[Abstract] In this paper we summarize the long-term effects of A-bomb radiation on the T-cell system and discuss the possible involvement of attenuated T-cell immunity in the disease development observed in A-bomb survivors. Our previous observations on such effects include impaired mitogen-dependent proliferation and IL-2 roduction, decreases in naive T-cell populations, and increased proportions of anergic and functionally weak memory CD4 T-cell subsets. In addition, we recently found a radiation dose-dependent increase in the percentages of $CD25^{+}/CD127^{-}$ regulatory T cells in the CD4 T-cell population of the survivors. All these effects of radiation on T-cell immunity resemble effects of aging on the immune system, suggesting that ionizing radiation might direct the T-cell system toward a compromised phenotype and thereby might contribute to an enhanced immunosenescence. Furthermore, there are inverse, significant associations between plasma levels of inflammatory cytokines and the relative number of naïve CD4 T cells, also suggesting that the elevated levels of inflammatory markers found in A-bomb survivors can be ascribed in part to T-cell immunosenescence. We suggest that radiation-induced T-cell immunosenescence may

result in activation of inflammatory responses and may be partly involved in the development of aging-associated and inflammation-related diseases frequently observed in A-bomb survivors.

Other Journal Publications

♦ Hayashi T, Kusunoki Y: Molecular genetics/epidemiology of cancer. Koshu Eisei [Public Health] 2010 (September); 74(9):738–43. (Japanese)

♦ Kusunoki Y, Yamaoka M, Kubo Y, Hayashi T, Kasagi F, Nakachi K: Effects of atomic-bomb radiation on human immune response. Report 25: A dose-dependent increase in the percentage of regulatory CD4 T cells. Nagasaki Igakkai Zasshi [Nagasaki Medical Journal] 2010 (September); 85(Special Issue):282–6. (Proceedings of the 51st Late A-bomb Effects Research Meeting, 2010) (Japanese)

♦ 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] 2010 (April); 63(4):345–7. (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] 2010 (April); 63(4):348–50. (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

Manuscript in Press

⌘ Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y: Lymphocyte subset characterization associated with persistent hepatitis C virus infection and subsequent progression of liver fibrosis. Human Immunology. (related to *Special Clinical Studies*)

Immunology Studies Oral Presentations

❖ Hayashi T, Morishita Y, Nagamura H, Maki M, Kusunoki Y, Yoshida K, Imai K, Cologne JB, Nakachi K. Genetic susceptibility to radiation-associated colon and rectum cancers among atomic-bomb survivors with special reference to the *CD14* gene. The 101st Annual Meeting of the American Association for Cancer Research (AACR), 17–21 April 2010, Washington DC, USA

❖ Hayashi T, Kusunoki Y, Morishita Y, Nagamura H, Maki M, Kubo Y, Yamaoka M, Hayashi I, Yoshida K, Imai K, Nakachi K. Attenuation of the immune system and elevated inflammatory markers caused by aging and atomic bomb radiation exposure. The 1st Annual World Congress of Immunodiseases and Therapy, 15–17 May 2010, Beijing, China

❖ Morishita Y, Nagamura H, Maki M, Kusunoki Y, Yoshida K, Imai K, Nakachi K, Hayashi T. Effects of *IL-10* gene polymorphisms on risk of radiation-associated cancers in atomic-bomb survivors. The 1st Annual World Congress of Immunodiseases and Therapy, 15–17 May 2010, Beijing, China

❖ Ohishi W, Yoshida K, Hayashi T, Kusunoki Y, Fujiwara S, Nakashima E, Tsuge M, Chayama K. Effects of *HLA-DRB1* and *NKG2D* polymorphisms on hepatitis C virus infection. The 46th Annual Meeting of the Japan Society of Hepatology, 27–28 May 2010, Yamagata (related to *Special Clinical Studies*)

❖ Kusunoki Y, Yamaoka M, Kubo Y, Hayashi T, Kasagi F, Nakachi K. Effects of atomic-bomb radiation on human immune response. Report 25: A dose-dependent increase in the percentage of regulatory CD4 T cells. The 51st Late A-bomb Effects Research Meeting, 6 June 2010, Nagasaki

❖ Kusunoki Y, Yamaoka M, Kubo Y, Imai K, Yoshida K, Ohishi W, Hayashi T, Nakachi K. Associations between *NKG2D* genotypes and inter-individual defferences in cell surface expression levels of NKG2D molecules in peripheral blood CD8 T and NK cell populations. The 20th Annual Meeting of the Japan Cytometry Society, 26–27 June 2010, Tokyo

❖ Hayashi T, Yoshida K, Morishita Y, Maki M, Sasaki K, Nagamura H, Imai K, Kusunoki Y, Nakachi K. Genetic susceptibility to radiation-associated colon and rectum cancers among atomic-bomb survivors with reference to the *CD14* gene. Scientific Meeting for Cancer Prevention 2010 Sapporo, 15–16 July 2010, Sapporo

❖ Hayashi T. Molecular epidemiology of lifestyle-related disease in relation to biological effects of cigarette smoking, using the data from a general population cohort study. FY2009 Research Meeting of Smoking Research Foundation, 28 July 2010, Tokyo

❖ Yoshida K, Ohishi W, Morishita Y, Nagamura H, Maki M, Sora M, Sasaki K, Chayama K, Fujiwara S, Kusunoki Y, Imai K, Nakachi K, Hayashi T. Effects of *NKG2D* and *MICA* genetic polymorphisms on hepatitis C virus infection. BIT's 1st World Congress of Virus and Infections 2010, 31 July–3 August 2010, Busan, South Korea (related to *Special Clinical Studies*)

❖ Hayashi T. Attenuation of the immune system caused by aging and atomic bomb radiation exposure. Research Seminar in National Cancer Center, Republic of Korea, 6 August 2010, Seoul, South Korea

❖ Geyer SM, Hayashi T, Misumi M, Furukawa K, Kusunoki Y, Nakachi K. Methods for development of an immune scoring system. The 14th International Congress of Immunology, 22–27 August 2010, Kobe

❖ Hayashi T, Morishita Y, Sora M, Yoshida K, Imai K, Hayashi I, Kusunoki Y, Nakachi K. High-throughput flow cytometric assay system of reactive oxygen species in blood cells. The 14th International Congress of Immunology, 22–27 August 2010, Kobe

❖ Kusunoki Y, Yoshida K, Hayashi T, Nakachi K. Increased T-cell populations with compromised/senescent phenotype in atomic-bomb survivors. The 14th International Congress of Immunology, 22–27 August 2010, Kobe

❖ Morishita Y, Hayashi T, Nagamura H, Maki M, Yoshida K, Cologne JB, Imai K, Kusunoki Y, Nakachi K. Joint effects of *HLA-DRB1* and *IL-10* gene polymorphisms on risk of radiation-associated gastric cancer in atomic-bomb survivors. The 19th Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics, 17–19 September 2010, Tokyo

❖ Hayashi T, Yoshida K, Imai K, Kusunoki Y, Nakachi K. Colon and rectum cancer risks of atomic-bomb survivors in relation to *CD14* gene polymorphisms and prior radiation

exposure dose. The 69th Annual Meeting of the Japanese Cancer Association, 22–24 September 2010, Osaka

❖ Hayashi T, Nakachi K, Yoshida K, Imai K, Cologne JB, Kyoizumi S, Kusunoki Y, Douple EB, Shore RE. Molecular epidemiology of radiation susceptibility to cancer among atomic-bomb survivors. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA

❖ Kusunoki Y, Kubo Y, Yamaoka M, Yoshida K, Hayashi T, Nakashima E. Increased percentages of both T_H1 and T_H2 cells in the peripheral blood lymphocyte population among A-bomb survivors. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y. Enhanced T_H1 immunity in association with persistent infection of hepatitis C virus and progression of liver fibrosis. Keystone Symposium "Immunologic Memory, Persisting Microbes and Chronic Disease," 6–11 February 2011, Banff, Canada (related to *Special Clinical Studies*)

❖ Hayashi T, Morishita Y, Nagamura H, Maki M, Cologne JB, Yoshida K, Imai K, Kyoizumi S, Kusunoki Y, Nakachi K. Genetic susceptibility to radiation-associated cancer occurred in atomic-bomb survivors. The 10th Korea-Japan Cancer and Aging Symposium, 14–15 February 2011, Tokyo

❖ Yoshida K, Ohishi W, Imai K, Maki M, Sasaki K, Chayama K, Kusunoki Y, Nakachi K, Hayashi T. Anticipation of natural course of HCV infection in terms of *NKG2D* and *IL28B* gene polymorphisms. The 10th Korea-Japan Cancer and Aging Symposium, 14–15 February 2011, Tokyo (related to *Special Clinical Studies*)

❖ Kusunoki Y, Yoshida K, Hayashi T, Imai K, Kyoizumi S, Nakachi K. Individual variation in radiation-induced mutant frequency at the erythrocyte *glycophorin A* and polymorphisms in DNA repair genes. The 33rd Annual Meeting of the Japan Society for Hematopoietic Cell Transplantation, 9–10 March 2011, Matsuyama

Research Protocols 3-11, 3-10, 2-10, 6-08, 4-08, 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 3-11 Radiation exposure in children and *in utero* survivors of the atomic bombing of Hiroshima and Nagasaki and late-life neurocognitive function

Yamada M (CH), Hida A (CN), Akahoshi M (CN), Kasagi F (EH), Abbott RD (S), Khattree R (S), Ohshita T, Miyachi T, Matsumoto M, Tsujino A, Mimori Y, Krull KR, Fujiwara S (CH)

We will examine late-life neurocognitive function among the Adult Health Study (AHS) subjects who were exposed *in utero* or aged <12 years at the time of the atomic bombing of Hiroshima and Nagasaki. Objectives, are (1) to examine associations between radiation exposure and neurocognitive function as assessed by neuropsychological examination, (2) to investigate the effects of sex, age, attained education, lifestyle, and comorbidity on neurocognitive function as risk factors or modifiers of radiation effects, and (3) to collect baseline data on cognitive function for the investigation of longitudinal progression in cognitive decline with advancing age and the occurrence of dementia. The Cognitive Abilities Screening Instrument (CASI) and the Childhood Cancer Survivors Study (CCSS)-Neurocognitive Questionnaire (NCQ) will be used as instruments for neuropsychological assessment.

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

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

The ophthalmological study of A-bomb survivors conducted between 2000 and 2002 based on RP 3-00 showed statistically significant dose-response relationship for subcapsular and cortical cataract. Furthermore, a lower dose threshold or no threshold is suggested. The objective of this addendum study is to further investigate the following unanswered questions of RP 3-00: (1) whether time-dependent progression is seen in radiation-induced cataract and (2) whether dose response relationship is observed when cataract cases are assessed using a radiation-specific classification method (Merriam-Focht method).

Study methods: (1) AHS participants who were 13 years old or younger at the time of atomic bombings will be studied. (2) Assessment will be done by ophthalmologists based on the Lens Opacities Classification System Version II (LOCS II) and Merriam-Focht method. (3) Various confounding factors will be taken into consideration at the time of analysis. (4) Digital images of the lens and retina will be stored.

In accordance with the above methods, the ophthalmological study started in Hiroshima and Nagasaki in August 2010.

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), 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 arteriosclerotic 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.

During the period of November 2008 and November 2010, we measured liver stiffness with the elastometer for 2,400 survivors, and the measurement has almost been completed for younger survivors (less than 10 years of age at the time of atomic bombings). We also measured blood cytokine levels related to chronic inflammation and/or insulin resistance for about 2,100 survivors.

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), Tatsukawa Y (CH), 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 Gy 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 December 2008 in Hiroshima and on November 2009 in Nagasaki. A total of 32 samples in Hiroshima and 4 samples in Nagasaki have been collected and stored as of March 2011.

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, 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 published in *Journal of Radiation Research* (Hsu et al., 2010; 51:431–9). Subsequently, a joint model to estimate causal associations among radiation, inflammation, and solid cancer incidence was specified and analyzed. A manuscript is now under internal review. 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)

Objectives: 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, and to determine if these factors also increased the risk of chronic inflammation and myocardial infarction.

Background and significance: The Life Span Study (LSS) and the Adult Health Study (AHS) cohort members were selected from among survivors alive in 1950; it has since been reported that a "healthy survivor effect" was suspected for non-cancer mortality among survivors. The hypothesis is that genetic polymorphisms controlling responsiveness to external stresses can alter survival rates following acute radiation exposures but also increase myocardial infarction in later life.

Study methods: A case-control study among younger survivors who participated at the first health examination in 1958–59 and were exposed to at least 1 Gy of radiation, and their sex-, age-, and city-matched controls. We will analyze the *LTA* single-nucleotide polymorphism (SNP) and *TRL2* genes among them.

Study progress: The genotyping (*LTA* and *TRL2* genes) is ongoing.

Results and conclusions: None yet.

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 has been 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), Tatsukawa Y (CH), 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. The preliminary results indicate that there was no association between parental radiation and any types of cataract in the offspring. Further analyses will be conducted.

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

The objectives of this study are 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 two 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, nine 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), Tatsukawa Y (CH), 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

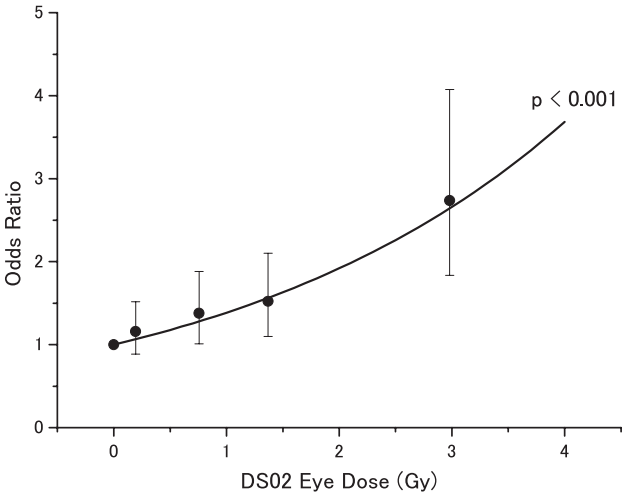

Figure. The main-effect model dose-response curve in regression analysis after adjustingfor city, sex, age at the time of the bombings, and diabetes mellitus in A-bomb survivors who had undergone lens removal surgery (OR at 1 Gy, 1.39; CI, 1.24–1.55).

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 four 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, Nakashima et al., *Annals of the Institute of Statistical Mathematics* 2008; 60[3]:465–82, and Minamoto et al., *Journal of Photochemistry and Photobiology B: Biology* 2011; 103[2]:105–10). 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. A manuscript has been completed and will soon be submitted for internal review.

The results regarding cataract surgery incidence from 1986 to 2005 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 (journal submission in preparation). A Workshop summary, including the current results, has been published in *Radiation Research* (Blakely et al., 2010; 173[5]:709–17).

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). 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.

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), Yoshida K (R), Kusunoki Y (R), Hayashi T (R), Akahoshi M (CN), Chayama K

The hypothesis behind this study is that radiation may increase the incidence of hepatocellular carcinoma either by increasing the rates of hepatitis B or C virus infection or by facilitating clinical 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 studied immunological characteristics regarding clinical progression following HCV infection, taking radiation exposure into account (RP 1-93) in collaboration with the Department of Radiobiology/Molecular Epidemiology. It was suggested that increased percentage of Th1 cells and decreased percentages of Tc1 cells and NK cells may be associated with progression of liver fibrosis among individuals with persistent HCV infection. The results will soon be published (Yoshida et al., *Human Immunology* 2011, in press).

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.

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 Neurological Sciences* 2009; 281:11–4 and Yamada et al., *Journal of Neurological Sciences* 2009; 283:57–61). We considered some instruments to use for assessing cognitive function and other psycho-neurological function among the younger and *in utero* survivors and prepared a new research protocol.

No association was found between previous radiation exposure and cognitive impairment and/or development of dementia among subjects exposed at ≥13 years of age. A new research protocol on radiation exposure in children and *in utero* survivors and late-life neurocognitive function has been submitted for internal (RERF) and external reviews.

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

Objectives: 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.

Background and significance: 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.
Study methods: Longitudinal follow-up study of BMD as a part of routine health examinations.
Study progress: We are conducting international and national collaboration studies using accumulated data and information related to this RP.
Results and conclusions: As a collaborative study with the WHO working group, we have published a Japanese version of the WHO fracture risk assessment tool including age, sex, BMD, prior fracture, smoking, alcohol drinking, and so on as risk factors (Fujiwara et al., *Osteoporosis International* 2008; 19:429–35). Our papers from the AHS have contributed to development of a WHO fracture risk assessment tool, Japanese guidelines for prevention and treatment, guidelines for treatment of steroid induced osteoporosis, and so on.

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 is 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 epidemiologic methodology developed by this project has indicated a weak, but very consistent association between radiation dose and various endpoints of atherosclerosis, including myocardial infarction, thromboembolic 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 have 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 began in 2010.

This study is helping the cardiovascular disease working group develop and confirm hypotheses regarding low-dose radiation effects on cardiovascular disease.

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

Neriishi K (CH), Takahashi I (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 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 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 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 11 cohorts in Europe, U.S.A., Australia, Brazil, and Japan (Nagasaki AHS). The objective of this study is to assess the relationship between subclinical thyroid dysfunction and coronary heart disease and mortality.

No constant results have been obtained from prospective cohort studies regarding the association between subclinical hypothyroidism and cardiovascular outcomes. These conflicting results might reflect differences in participants' age, gender, thyroid-stimulating hormone (TSH) levels or preexisting cardiovascular disease.

We performed an individual participant data analysis of 55,287 participants (542,494 person-years) in 11 prospective cohort studies. We examined the risk of coronary heart disease (CHD) events and CHD mortality in all cohorts. With subclinical hypothyroidism, CHD risks did not significantly differ by age, gender, or preexisting cardiovascular disease. At the same time, subclinical hypothyroidism is associated with an increased risk of CHD events and CHD mortality in those with higher TSH levels, especially those with TSH ≥10 mIU/L (Rodondi et al., *JAMA* 2010; 304[12]:1365–74).

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 during 1990–2007 (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 males) 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)

♦ 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 2010 (May); 72(5):689–95.

 (RR 15-08)

[Abstract] ***Objective:*** A possible association between subclinical hypothyroidism and cardiovascular disease (CVD) has been reported. Monitoring of atomic-bomb survivors for late effects of radiation exposure at the Radiation Effects Research Foundation has provided the opportunity to examine associations between subclinical hypothyroidism and metabolic CVD risk factors. The objective of the study was to evaluate associations between subclinical hypothyroidism and metabolic CVD risk factors, and a cluster of these factors. ***Design and participants:*** This was a cross-sectional study of 3,549 subjects (mean age 70 years; 1,221 men and 2,328 women) between 2000 and 2003 comprising 306 subjects with subclinical hypothyroidism and 3,243 control euthyroid subjects in Japan. ***Measurements:*** We investigated associations between subclinical hypothyroidism and metabolic CVD risk factors such as hypertension, diabetes mellitus, dyslipidaemia and hyperuricaemia, and a cluster of these factors. ***Results:*** Subclinical hypothyroidism was not significantly associated with either hypertension, diabetes mellitus or hyperuricaemia defined by taking into account the use of medications in both men and women, but in men it was associated with dyslipidaemia (P = 0.02). We observed a significantly increased odds ratio (OR) for the presence of three or more metabolic CVD risk factors in men with subclinical hypothyroidism after adjusting for age, body mass index (BMI), and smoking status [OR: 1.83, 95% confidence interval (CI): 1.13–2.94, P = 0.01]. The significant associations remained after an additional adjustment for atomic-bomb radiation dose. ***Conclusions:*** There appears to be a significant increase in a cluster of metabolic CVD risk factors among people with subclinical hypothyroidism.

♦ 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 2010 (July); 51(4):431–9. (RR 5-09) (refer to abstract in *Adult Health Study* Publications)

♦ Shore RE, Neriishi K, Nakashima E: Epidemiological studies of cataract risk at low to moderate radiation doses: (Not) seeing is believing. Radiation Research 2010 (December); 174(6):889–94. (RR 9-10) (refer to abstract in *Adult Health Study* Publications)

Commentary and Review Series (CR)

♦ 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 2010 (April); 33(4):638–43.

 (CR 4-09)

[Abstract] Although fatty liver predicts ischemic heart disease, the incidence and predictors of fatty liver need examination. The objective of this study was to determine fatty liver incidence and predictive variables. Using abdominal ultrasonography, we followed biennially through 2007 (mean follow-up, 11.6 ± 4.6 years) 1,635 Nagasaki atomic bomb survivors (606 men) without fatty liver at baseline (November 1990 through October 1992). We examined potential predictive variables with the Cox proportional hazard model and longitudinal trends with the Wilcoxon rank-sum test. In all, 323 (124 men) 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, and smoking and drinking habits, obesity (relative risk [RR], 2.93; 95% confidence interval [CI], 2.33–3.69, $P < 0.001$), low high-density lipoprotein-cholesterol (RR, 1.87; 95% CI, 1.42–2.47; $P < 0.001$), hypertriglyceridemia (RR, 2.49; 95% CI, 1.96–3.15; $P < 0.001$), glucose intolerance (RR, 1.51; 95% CI, 1.09–2.10; $P = 0.013$) and hypertension (RR, 1.63; 95% CI, 1.30–2.04; $P < 0.001$) were predictive of fatty liver. In multivariate analysis including all variables, obesity (RR, 2.55; 95% CI, 1.93–3.38; $P < 0.001$), hypertriglyceridemia (RR, 1.92; 95% CI, 1.41–2.62; $P < 0.001$) and hypertension (RR, 1.31; 95% CI, 1.01–1.71; $P = 0.046$) 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. Obesity, hypertriglyceridemia and, to a lesser extent, hypertension might serve as predictive variables for fatty liver.

Other Journal Publications

♦ Blakely EA, Kleiman NJ, Neriishi K, Chodick G, Chylack LT, Cucinotta FA, Minamoto A, Nakashima E, Kumagami T, Kitaoka T, Kanamoto T, Kiuchi Y, Chang P, Fujii N, Shore RE: Meeting report: Radiation cataractogenesis: Epidemiology and biology. Radiation Research 2010 (May); 173(5):709–17.

♦ Fujiwara S: Importance of raising awareness about spontaneous insufficiency fractures in the bedridden elderly: International Journal of Clinical Rheumatology 2010 (August); 5(4):395–7.

♦ Thyroid Studies Collaboration (RERF: Imaizumi M): Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA 2010 (September); 304(12):1365–74.

♦ Yamada M: Follicle stimulation hormone and estragiol levels during perimenopause in a cohort of Japanese women: The Radiation Effects Research Foundation Adult Health Study. Michalski J, Nowak I, eds. Menopause: Vasomotor Symptoms, Systematic Treatments and Self-Care Measures. New York: Nova Science Publishers; 2010, pp 112–24.

♦ Yamada M, Imaizumi M, Ohishi W: Thyrotropin levels during the perimenopause: The Radiation Effects Research Foundation Adult Health Study. Michalski J, Nowak I, eds. Menopause: Vasomotor Symptoms, Systematic Treatments and Self-Care Measures. New York: Nova Science Publishers; 2010, pp 125–31.

Manuscripts in Press

⌘ Fujiwara S, Hamaya E, Goto W, Masunari N, Furukawa K, Fukunaga M, Nakamura T, Chen P: Vertebral fracture status and the World Health Organization risk factors for predicting osteoporotic fracture risk in Japan. Bone.

⌘ Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M: Incidence and prognostic value of early repolarization pattern in the 12-lead electrocardiogram. Circulation.

⌘ Minamoto A, Neriishi K, Nakashima E: UV radiation may explain intercity difference for cataract in A-bomb survivors. Journal of Photochemistry and Photobiology B: Biology.

⌘ Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y: Lymphocyte subset characterization associated with persistent hepatitis C virus infection and subsequent progression of liver fibrosis. Human Immunology. (related to *Immunology Studies*)

Special Clinical Studies Oral Presentations

❖ Tsuneto A, Hida A, Sera N, Imaizumi M, Ichimaru S, Nakashima E, Seto S, Eishi K, Maemura K, Akahoshi M. Fatty liver incidence and predictive variables. The 107th Annual Meeting of the Japanese Society of Internal Medicine, 9–11 April 2010, Tokyo

❖ Rodondi N, den Elzen W, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Maisonneuve P, Bremner A, Vanderpump M, Newman AB, Cornuz J, Franklyn JA, Westendorp RGJ, Vittinghoff E, Gussekloo J for the Thyroid Studies Collaboration. Subclinical hypothyroidism and the risk of coronary heart disease and mortality: An individual participant pooled analysis from nine cohort studies. The 33rd Annual Meeting of the Society of General Internal Medicine (SGIM), 28 April–1 May 2010, Minneapolis, Minnesota, USA

❖ Fujiwara S. Epidemiology of osteoporotic vertebral fractures. The 83rd Annual Congress of Japanese Orthopaedic Association, 26–30 May 2010, Tokyo

❖ Ohishi W, Yoshida K, Hayashi T, Kusunoki Y, Fujiwara S, Nakashima E, Tsuge M, Chayama K. Effects of *HLA-DRB1* and *NKG2D* polymorphisms on hepatitis C virus infection. The 46th Annual Meeting of the Japan Society of Hepatology, 27–28 May 2010, Yamagata (related to *Immunology Studies*)

❖ Tatsukawa Y, Masunari N, Ohishi W, Yamada M, Yamane K, Fujiwara S. Relationship between nonalcoholic fatty liver disease and prevalence of diabetes and metabolic syndrome. The 53rd Annual Scientific Meeting of the Japan Diabetes Society, 27–29 May 2010, Okayama

❖ Fujiwara S, Masunari N, Yamane K, Makiguchi M, Fukunaga M. Prediction of hip fracture risk based on hip geometry parameters in the Hiroshima cohort. The 37th European Symposium on Calcified Tissues, 26–30 June 2010, Glasgow, UK

❖ Yamada M, Kasagi F, Mimori Y, Miyachi T, Oshita T, Sasaki H. Grip strength as a predictor of mortality and dementia: Radiation Effects Research Foundation Adult Health Study. Alzheimer's Association International Conference on Alzheimer's Disease 2010, 10–15 July 2010, Honolulu, Hawaii, USA

❖ Fujiwara S, Masunari N, Ohishi W. Prediction of bone loss rates and bone fracture risk using bone metabolism markers and serum undercarboxylated osteocalcin (ucOC): Hiroshima cohort study. The 28th Conference of Japanese Society of Bone and Mineral Metabolism, 21–23 July 2010, Tokyo

❖ Fujiwara S. Diagnosis and treatment of osteoporosis—Definition of osteoporosis-induced bone-fracture risk and time of initiation of treatment. The 138th Symposium of the Japanese Association of Medical Sciences, 29 July 2010, Tokyo

❖ Yoshida K, Ohishi W, Morishita Y, Nagamura H, Maki M, Sora M, Sasaki K, Chayama K, Fujiwara S, Kusunoki Y, Imai K, Nakachi K, Hayashi T. Effects of *NKG2D* and *MICA* genetic polymorphisms on hepatitis C virus infection. BIT's 1st World Congress of Virus and Infections 2010, 31 July–3 August 2010, Busan, South Korea (related to *Immunology Studies*)

❖ Neriishi K, Nakashima E, Minamoto A. Localization of lens opacity and model fitting analysis by UV substitution in cataract of A-bomb survivors. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA

❖ Shore RE, Ozasa K, Kasagi F, Imaizumi M, Tatsukawa Y. Are those exposed *in utero* the most radiosensitive population?—The Japanese A-bomb experience. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA (related to *Life Span Study* and *Adult Health Study*)

❖ Tatsukawa Y, Masunari N, Yamada M, Fujiwara S. Effects

of metabolic syndrome and hypertension on incidence of peripheral artery disease: Adult Health Study in Hiroshima. The 23rd Scientific Meeting of the International Society of Hypertension, 26–30 September 2010, Vancouver, Canada

❖ Fujiwara S. Epidemiology of osteoporosis and related fracture. The 12th Annual Meeting of the Japan Osteoporosis Society, 21–23 October 2010, Osaka

❖ Fujiwara S. Evaluation of standardised SOS/BUA cut-off values for bone fracture. The 12th Annual Meeting of the Japan Osteoporosis Society, 21–23 October 2010, Osaka

❖ Fujiwara S. Lifestyle diseases and fracture frequency: Lifestyle diseases as fracture risk factors. The 12th Annual Meeting of the Japan Osteoporosis Society, 21–23 October 2010, Osaka

❖ Imaizumi M. Thyroid diseases among A-bomb survivors in Hiroshima and Nagasaki. The 53rd Annual Meeting of the Japan Thyroid Association, 11–13 November 2010, Nagasaki

❖ Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M. Prevalence, incidence and prognostic value of early repolarization pattern. American Heart Association Scientific Sessions 2010, 13–17 November 2010, Chicago, Illinois, USA

❖ Neriishi K. Current status of cataract study of A-bomb survivors. Nagasaki University Global COE Program "International Symposium on Radiation Epidemiology and Radiobiology," 29–30 November 2010, Nagasaki

❖ Fujiwara S. Loss of body height and osteoporotic fracture. Joint Symposium, Korean Osteoporosis Society and Japan Osteoporosis Society, 4–5 December 2010, Seoul, South Korea

❖ Shore RE. Cardiovascular risk after low-dose radiation exposure: A timely question. Workshop: Planning Pilot Study of One Million U.S. Radiation Workers, 3 February 2011, Oak Ridge, Tennessee, USA (related to *Life Span Study*)

❖ Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y. Enhanced T_H1 immunity in association with persistent infection of hepatitis C virus and progression of liver fibrosis. Keystone Symposium "Immunologic Memory, Persisting Microbes and Chronic Disease," 6–11 February 2011, Banff, Canada (related to *Immunology Studies*)

❖ Yoshida K, Ohishi W, Imai K, Maki M, Sasaki K, Chayama K, Kusunoki Y, Nakachi K, Hayashi T. Anticipation of natural course of HCV infection in terms of *NKG2D* and *IL28B* gene polymorphisms. The 10th Korea-Japan Cancer and Aging Symposium, 14–15 February 2011, Tokyo (related to *Immunology Studies*)

❖ Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M. Prevalence, incidence and prognostic value of early repolarization pattern. The 75th Annual Meeting of the Japanese Circulation Society, 18–20 March 2011, Yokohama

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 surgically removed 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 of the 648 cases, we found 595 cases were MPC, 41 cases (7%) were not MPC cases, 10 cases were undetermined and 2 cases were uncollectible.

Histopathology Study Oral Presentation

❖ Suyama A, Ozasa K, Soda M, Kodama K, Sekine I, Tokuoka S. Patho-epidemiological study in RERF. Nagasaki University Global COE Program "International Symposium on Radiation Epidemiology and Radiobiology," 29–30 November 2010, Nagasaki

Research Protocols 5-10, 5-02
Cell Biology Study

RP 5-10 Analyses of molecular characteristics of colorectal cancer among atomic-bomb survivors

Ito R (R), Hamatani K (R), Taga M (R), Imai K (R), Kasagi F (EH), Ozasa K (EH), Katayama H (IT), Cologne JB (S), Misumi M (S), Izumi S, Oue N, Yasui W, Nakachi K, Kusunoki Y (R)

Certain solid cancers show a significant and relatively high excess relative risk (ERR) from radiation exposure in the Life Span Study (LSS) of atomic-bomb (A-bomb) survivors. Those victims who had A-bomb exposure at a young age (0–19 years old) show a higher ERR of solid cancers, even 60 years after exposure, than do those exposed at over 20 years of age. Pathological studies have in the past provided significant information about the histological characteristics of various solid cancers that developed among A-bomb survivors, some of which seem to differ from those in the non-exposed population.

Although little has been proved about the molecular characteristics of solid cancers among A-bomb survivors, preliminary results obtained from our pilot study on colon and rectal cancers among A-bomb survivors implied that prior atomic radiation exposure might influence microsatellite instability (MSI) status and result in an increase in the relative frequency of MSI-high (MSI-H) colon cancer. In addition, MSI-related molecular events, typically genetic and epigenetic alterations of the *MLH1* and Ras-signaling-related genes, might also be influenced by radiation exposure.

Determination of colon and rectal cancers with MSI or chromosomal instability (CIN) is an important issue that forms the basis of this study. MSI status will be examined by DNA fragment analysis using six different microsatellite markers. In addition, CIN status will be examined by analysis of gain or loss of CIN-related chromosome loci with real-time polymerase chain reaction (PCR) or the PCR-restriction fragment length polymorphism (PCR-RFLP) method using single nucleotide polymorphism (SNP) loci, respectively.

Based on findings obtained from the pilot study, we propose the following hypothesis: The carcinogenic pathway with MSI may preferentially occur in colon cancer, but not in rectal cancer, among A-bomb survivors. To test this hypothesis, the following questions will be clarified by examination of about 140 cases with colon or rectal cancers including 20 subjects exposed to high radiation dose: 1) Whether radiation exposure has a stronger relationship with MSI-H colon cancer than with microsatellite stable/MSI-low (MSS/MSI-L) colon cancer or overall rectal cancer; 2) If this is the case, is radiation exposure associated with the occurrence of genetic and epigenetic alterations, specifically methylation of MSI-related genes (DNA repair genes, Ras-signaling-related genes, CpG island methylator phenotype [CIMP]-related genes) in colon cancer?; 3) Whether radiation exposure is associated with CIN-positive colon and rectal cancers; 4) If answer to question 3) is yes, whether radiation exposure influences the most important initial event (i.e., *adenomatous polyposis coli* [*APC*] gene alteration) or subsequent events; and 5) Are there any differences in pathological parameters (e.g., differentiation, development, progression, and host immune reaction to cancer) between

non-exposed and exposed MSI- or CIN-positive colon and rectal cancers?

In this study, we will examine those questions in the order of descending priorities indicated by the item numbers 1) to 5).

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), Soda M (EN), Katayama H (IT), Cologne JB (S), Hayashi Y, Nakachi K, Kusunoki Y (R)

Thyroid cancer is one of the malignancies most closely associated with radiation in humans. We have previously found that rearrangement of *RET* proto-oncogene could be induced in human thyroid cells by *in vitro* and *in vivo* X-ray irradiation. *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 an 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/PTC* rearrangements and other chromosomal rearrangements induced by 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) tended to increase with increase of radiation dose and numbered 25, in 10 of which we recently found rearranged anaplastic lymphoma kinase (*ALK*) gene for the first time in PTC. All of the 10 cases were exposed PTCs, but no *ALK* rearrangements were found in the six non-exposed PTC cases.

Trabecular/solid-like architectures were observed in six of the 10 PTC cases with *ALK* rearrangements, while such architectures were found in only two of the remaining 15 PTC cases without *ALK* rearrangements, indicating close association between trabecular/solid-like architectures in PTCs and *ALK* rearrangements. These PTC cases with trabecular/solid-like architectures and *ALK* rearrangements comprised heavily exposed survivors with younger age ATB and younger age at diagnosis, compared with the other PTC cases. The findings suggest the possibility that *ALK* rearrangements as well as *RET/PTC* rearrangements may play crucial roles in adult-onset PTC.

Cell Biology Study Oral Presentations

❖ Hamatani K, Mukai M, Hayashi Y, Takahashi K, Nakachi K, Kusunoki Y. Rearranged anaplastic lymphoma kinase (*ALK*) gene found for the first time in adult-onset papillary thyroid cancer cases among atomic bomb survivors. The 14th International Thyroid Congress, 11–16 September 2010, Paris, France

❖ Hamatani K, Takahashi K, Ito R, Taga M, Niwa Y, Nakachi K, Kusunoki Y. Rearrangement of *ALK* gene found in papillary thyroid cancer cases with high radiation dose and/or short latency. The 69th Annual Meeting of the Japanese Cancer Association, 22–24 September 2010, Osaka

❖ Takahashi K, Taga M, Ito R, Niwa Y, Hayashi Y, Nakachi K, Kusunoki Y, Hamatani K. Rearrangement of *ALK* gene found for the first time in papillary thyroid cancer among atomic-bomb survivors. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

Research Protocols 1-11, 1-10, 2-07, 1-07, 1-97 and 2-01, 5-85 and 1-01

Biochemical Genetics Studies

RP 1-11 Study of radiation-induced circulatory diseases using animal models

Takahashi N (G), Niwa Y (R), Ohishi W (CH), Hayashi T (R), Murakami H (G), Hsu WL (S), Kokubo T, Inaba T, Oghiso Y, Tanaka S, Kusunoki Y (R)

The Life Span Study (LSS) data indicate radiation-associated risk for hypertensive heart disease and stroke, and the Adult Health Study (AHS) data suggest radiation-associated risk for hypertension. We hypothesize that radiation may result in higher risk for circulatory diseases (CD). However, there are a limited number of animal model studies that have examined the relationship between radiation and CD at doses under 4 Gy, although some papers report that various biomarkers are affected by radiation. In this study, we propose the use of spontaneous hypertension rat-stroke prone (SHRSP) rats as rat CD models. Radiation doses from a brief single exposure will be given to the rats at 4, 2, and 1 Gy, with non-exposed (0 Gy) rats used as controls. We will evaluate acceleration of the development of hypertension in the SHRSP rats after radiation exposure. Moreover, we will measure candidate serum markers that have shown radiation dose effects based on previous AHS studies. In addition to serum markers, we will also measure 23 biomarkers in plasma samples by immunoassay. The priority of each plasma marker will be determined on the basis of previous results from AHS studies, and we plan to examine the effects of radiation exposure in morphological types in autopsy tissues by collaborating with the Institute for Environmental Sciences. This study will provide mechanistic information on the association between radiation exposure and development of CD.

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)

The purpose of the study is to estimate the mutation induction rate following 4 Gy of gamma irradiation of mouse spermatogonia, and to molecularly characterize the mutations as an animal model of human male exposure. Based on recent advances in the comparative genomic hybridization (CGH) microarray technology, the RERF Scientific Council recommended that RERF conduct a pilot study using HD-array CGH with mouse and rat DNA samples that were used for DNA 2-DE studies. This pilot study will provide crucial information necessary for planning future genetic studies. Interpretation of the results will be important in order to determine the feasibility of RERF examining the DNA obtained (e.g., whether or not duplications occur as frequently as deletions) from the offspring of Hiroshima and Nagasaki survivors whose radiation doses are much smaller than those used in animal studies.

We will estimate the mutation induction rate by examining DNA samples of 30 F_1 mice derived from the spermatogonia of male mice irradiated with 4 Gy of γ-rays and 30 F_1 mice in the control group by a HD-array CGH technique. To detect small deletions harboring only 2–3 probes accurately, we have improved the experimental and analytical methods for the CGH platform. We finished the CGH experiment on the DNA samples of 30 exposed and 30 control mice. We will analyze the data to identify mutation candidates and will confirm the putative mutations molecularly.

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).

In addition to rare CNVs, we summarize the data by focusing on highly polymorphic CNVs (in ≥5% of the individuals). We found a total of 680 CNVs at 16 different BAC-regions in the genome. Some of the CNVs contained genes which might be useful markers to study the relationship between the genotypes and phenotypic heterogeneity among individuals. These results were published in *Journal of Biomedicine and Biotechnology* (Takahashi et al., Volume 2011, Article ID 820472).

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 (ATPase, Ca++ transporting, plasma membrane 1 [*ATP2B1*]) was significantly associated with hypertension. Odds ratio is 1.31 ($p = 4.1 \times 10^{-11}$). 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. The expression analysis of mRNA of this enzyme could also validate this result. On the other hand, we confirmed polymorphic genetic variations in three genes to be associated with blood pressure levels and risk of hypertension. 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 (RP 5-85) 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.

In addition to this effort, we started to collect untreated blood samples for future genetics studies with new technologies, such as a next generation sequencer. We collected 13 new blood samples from F_1 Clinical Study participants (as a part of RP 4-10).

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.

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	609	46	1,044
Unknown	0	7	30	0	37
Total	116	374	984	57	1,531

Biochemical Genetics Studies Publications

Journal Publications

♦ Global Blood Pressure Genetics Consortium (RERF: Takahashi N): Common variants in the ATP2B1 gene are associated with susceptibility to hypertension. The Japanese millennium genome project. Hypertension 2010 (November); 56(5):973–80. (related to *Adult Health Study*)

♦ Takahashi N, Satoh Y, Sasaki K, Shimoichi Y, Sugita K, Katayama H: Characteristics of highly polymorphic segmental copy-number variations observed in Japanese by BAC-array-CGH. Journal of Biomedicine and Biotechnology 2011 (January); Volume 2011(Article ID 820472):1–11.

Biochemical Genetics Studies Oral Presentations

❖ Asakawa J, Kamiguchi Y, Kaneoka S, Nakamoto Y, Tsuji T, Mishima S, Miura A, Kaneko J, Haba H, Imanaka M, Katayama H, Cullings HM, Kamiya K, Nakamura N. Estimation of genetic risk of radiation on immature oocytes of rats by using two-dimensional DNA analysis: An animal model for human female exposure. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Kodaira M, Nakamoto Y, Miura A, Tsuji T, Nishimura M, Shimada Y, Furukawa K, Cullings HM, Asakawa J. Estimation of genetic effects of radiation with high-density microarray CGH platform (2): An exploratory study using 720K-array format. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Tsuji T, Miura A, Nakamoto Y, Kodaira M, Nakamura N, Asakawa J. Estimation of genetic effects of radiation with high-density microarray CGH platform (1): Improvement of sensitivity in detection of deletions. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Takahashi N, Satoh Y, Sasaki K, Shimoichi Y, Sugita K, Katayama H. Characteristics of highly polymorphic segmental copy-number variations (CNVs) observed in Japanese by BAC-array-CGH. The 33rd Annual Meeting of the Molecular Biology Society of Japan, 7–10 December 2010, Kobe

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 screened DNA from 502 microscopic slides (that were used for the F_1 cytogenetic study) and 40 nonmelanoma skin cancers. We found five XPA heterozygotes among 502 ordinary people and none among the 40 skin cancers. The frequency of ordinary people is similar to that of the previous study (Hirai et al., *Mutation Research* 2006; 601:171–8).

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)

(1) Last year we reported the generation of HPRT-dupGFP mice. However, it turned out that *in vivo* observations were problematic as we could not detect any mutant cells in the mouse tissues (testis, liver, kidney, brain, etc). That was probably because tissue autofluorescence is relatively high, while expression of HPRT-GFP proteins is very low, in mutant cells. Thus a stronger promoter for the fusion protein expression seemed to be necessary for utilizing this mutant assay system. This year we have tried to replace endogenouse HPRT promoter with a much stronger CAG promoter (combined promoter with Cytomegarovirus enhancer/β-Actin promoter/β-Blobin intron carrying another enhancer) in HPRT-dupGFP ES cells. Thus, second targeting of the ES cells was carried out, and we successfully isolated the double knock-in cells. Upon a radiation-induced, or spontaneously arising reversion at the HPRT-dupGFP allele, the mutant ES cells exhibited >10 fold stronger fluorescence than those of former HPRT-dupGFP cells. Then we made mice with the newly generated ES

cells. This year we confirmed the successful generation of the HPRT^{dupGFP} mice. We now see brilliant mutant cells in mouse *in vivo* tissues such as in liver, pancreas, small intestine, lymphocytes, testis, etc.
(2) As reported last year, a conventional tet-regulated gene expression system (CMV-tetO-GFP/ CMV-TetR/floxed MCneo) did not work well in mouse ES cell. We thus made the TetO and TetR vectors with CAG promoter. ES cells bearing those vectors were generated (random integration of CAG-tetO-GFP, and targeted integration of CAG-TetR at the HPRT locus). GFP fluorescence upon mutations at the HP-tetR-RT locus was evaluated, and candidate ES cell clones for making mouse aggregation chimera were selected. This year, the CAG-TetR knock-in mice were generated.

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.

We examine the feasibility of specific single nucleotide polymorphisms (SNPs) that were suggested as contributing to the early-onset breast cancers. In this fiscal year, we examined MDM2 SNP309(G/T) among about 300 breast cancer cases in Hiroshima. A significant difference in the frequency of GG genotype was observed in the group A (A-bomb survivor, early onset) compared to other groups.

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)

Biodosimetric study using FISH

We examined 88 survivors (52 from Hiroshima and 36 from Nagasaki) by FISH. We also investigated clonal chromosome aberrations among translocations observed in 163 survivors (88 from Hiroshima and 75 from Nagasaki) who were examined by FISH in 2008–2010. We found 13 clonal aberrations in 9 survivors in Hiroshima and 7 clonal aberrations in 7 survivors in Nagasaki. The sizes of the clones were 1.2–15.8% (6–79/500 cells). Each of the clonal aberrations is counted as one event for calculation of the total aberration frequency.

Puzzling cytogenetic results for survivors exposed *in utero*—animal experiments

We have previously found that survivors who were exposed *in utero* showed almost no dose response in the translocation frequency in blood lymphocytes when examined at age 40, which was confirmed by subsequent mouse experiments. In order to see whether the above finding is unique in hematopoietic cells, we examined chromosome aberration frequencies in rat mammary epithelial cells following fetal irradiation. The results indicated that irradiated fetal mammary tissue recorded radiation damage like their mothers when examined at 6–45 weeks after irradiation, whereas lymphocytes did not record the damage. Thus, it is quite clear that the lack of translocation dose response following fetal exposure is tissue-dependent. A paper describing the results is under preparation.

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)

A long time after exposure to ionizing radiation (up to six months) we are able to successfully detect unrepairable DSB-foci in a dose dependent manner in normal human fibroblasts. We analyzed the role of poly-ubiquitination in the constituents of the foci for maintaining the architecture of the foci. Recombinant human cells producing bait proteins, such as TAP-tagged-Rap80, Abraxas, BRCC36, all of which were reported to be the focus components, were generated for isolating the unrepairable DSB-foci. So far, we have not found a difference between repairable DSB-foci and unrepairable DSB-foci by immunoprecipitation experiments. However, we found that the unrepairable large foci include some of the heterochromatin components as well as gene silencing factors.

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 either 0 Gy or 1 Gy of X-rays *in vitro*, and about 30 T-cell colonies of single-cell origin each in the 0-Gy and 1-Gy groups were collected. Cells from those T-cell colonies were sent to NIHS. CGH analyses with the DNA extracted from the cells are underway.

Cytogenetics Studies Publication

RERF Report (RR)

♦ Noda A, Hirai Y, Kodama Y, Kretzschmar WW, Hamasaki K, Kusunoki Y, Mitani H, Cullings HM, Nakamura N: Easy detection of GFP-positive mutants following forward mutations at specific gene locus in cultured human cells. Mutation Research 2011(March); 721(1):101–7.

 (RR 11-10)

[Abstract] We have generated a new mutation assay system using HT1080 human fibrosarcoma cells, which consists of a combination of tetracycline-operator dependent *GFP* gene (TetO-EGFP) and tetracycline repressor (*TetR*) genes, where the expression of *GFP* gene is under strict control of TetR protein, and the *TetR* gene is located within the endogenous *HPRT* gene. In this system, any inactivating mutation at the *TetR* gene or large deletions including the gene itself results in high expression of *GFP* gene (>200-fold increase) in the cells, which can be readily scored not only by a flow cytometer but also under a fluorescent microscope. With this new cell line, we show that the spontaneous mutation rate at the *TetR* locus was $2.8–3.4\times10^{-6}$/cell division, slightly lower than the rate at the endogenous *HPRT* gene of HT1080 cells, and has a dose response to X rays as a mutagen. We also isolated variant clones with elevated spontaneous mutation rate (i.e., genetically unstable cells) following X irradiation. Spontaneous GFP positive mutants were predominantly base-change mutations at the *TetR* gene while those obtained after X irradiation often contained large deletions which spanned up to 6 Mb. The results indicate that the bacterial TetR/TetO regulatory units work extremely well as a mutation detection system in human cells, and any part of the human genome may be tested for mutation sensitivity following targeted insertion of the *TetR* gene in a stably expressing gene.

Cytogenetics Studies Oral Presentations

❖ Noda A, Hirai Y, Kodama Y, Nakamura N. Generation of recombinant mice in which the living mutant cells fluoresce. The 35th Annual Meeting of the Chugoku Area Radiation Research Society, 26 July 2010, Hiroshima

❖ Nakamura N, Nakano M, Ohtaki K, Shimada Y, Kodama Y. Fetuses are not little children: Just ask their hematopoietic stem cells. The 56th Annual Meeting of the Radiation Research Society, 25–29 September 2010, Maui, Hawaii, USA

❖ Kodama Y, Nakano M, Ohtaki K, Hamasaki K, Shimada Y, Nishimura M, Yoshida M, Nakata A, Nakamura N. The lack of translocation dose response following fetal exposure is tissue-dependent. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Noda A, Oomine H, Hirai Y, Nakamura N, Kodama Y. Formation of heterochromatic structures at unrepairable and untethered broken double stand breaks. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Noda A, Hirai Y, Kodama Y, Nakamura N. Development of TetO-GFP/TetR cell system that easily detects forward mutations at specific gene locus through GFP gene expression. The 39th Annual Meeting of the Japanese Environmental Mutagen Society, 16–17 November 2010, Tsukuba

❖ Kodama Y. Biological dosimetry study of atomic bomb survivors in Radiation Effects Research Foundation. NIRS-IAEA Workshop on Cytogenetic Biodosimetry for Asia 2011, 26–27 January 2011, Chiba

❖ Kodama Y. Biodosimetric study in the atomic bomb survivors. The 13th Coordination and Planning Meeting of the WHO REMPAN Collaborating Centres and Liaison Institutions, 16–18 February 2011, Nagasaki

❖ Noda A, Oomine H, Hirai Y, Nakamura N, Kodama Y. Role of unrepairable DNA double strand breaks for determining cell fate long time after irradiation. The 1st International Symposium of Research Institute for Radiation Biology and Medicine, Hiroshima University, 3–4 March 2011, Hiroshima

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 through 2003 and will begin analyses of cancer incidence follow-up through 2005 by estimating the population for the F_1 cancer incidence study in the same way as for the LSS cancer incidence analysis.

F_1 Studies Publication

Journal Publication

♦ Suyama A: Children of atomic-bomb survivors. Nagasaki Association for Hibakusha's Medical Care (NASHIM), ed. Hibakusha in the 21st Century—Hibakusha in the World and Forefront of Study on Radiation Diseases. Nagasaki: Nagasaki Press; 2011 (March), pp 54–61. (Japanese)

Research Protocols 2-09, 1-09, 5-08 and 6-10, 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, A5-10, A3-10, 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.

A database has been 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, and analyses conducted.

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, 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 results indicated that radiation exposure was associated with increased risk of non-B, non-C HCC, independent of alcohol consumption, smoking, and BMI (Ohishi et al., *Hepatology* 2011, in press).

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). Using the ELISA method or the multiplex Luminex method, measurement of blood cytokine levels such as TNF-α, IL-6, MCP-1, leptin, and resistin was completed for 1,372 samples from HCC cases and controls under consideration.

RP 5-08 Breast cancer incidence among atomic-bomb survivors, 1950–2005
RP 6-10 Intrinsic subtypes of breast cancer among atomic-bomb survivors (Addendum to RP 5-08)

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). Of 2,116 possible breast cancer cases identified in the LSS cohort during 1950–2005, 1,732 cases were identified for histopathological review, and sample collection and pathologists' review were initiated. An 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 will be conducted for applicable cases (RP 6-10). After completing the histological reviews, the risk of breast cancer for radiation will be evaluated within histological classes. 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.

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. Among 4,318 possible cases, 136 cases were histologically diagnosed by pathologists as tumors of soft tissue and bone. Final consensus on diagnoses will be formed.

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. Therefore, in this study, the association between estimated radiation doses (DS02) and histologically confirmed corpus cancer and its sub-types will be investigated. Based on cancer registry information and other data, records of about 1,600 cases of possible uterine corpus cancer were reviewed. Of this total, about 400 cases were identified as requiring pathological review. Preparations for material collection are being made.

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)

Objectives: To determine whether the radiation exposure-dependent gastric cancer risk seen in the atomic-bomb survivors is related to chronic tissue inflammation associated with *H. pylori* infection.

Background and significance: Three major factors—environmental factors (diet, smoking), host factors (age, *H. pylori* infection), and genetic factors—jointly affect the genesis of gastric cancer. We will investigate interactions between radiation exposure and these risk factors in developing gastric cancer.

Study methods: A nested case-control study was performed in the longitudinal cohort of atomic-bomb survivors using stored sera before diagnosis. Enrollees included about 300 cancer cases and 3 controls per case selected from cohort members matched on age, gender, city, time of serum storage, and type of serum storage, and counter-matched on radiation dose.

Study progress: A manuscript on chronic gastritis and development of gastric cancer in relation to radiation exposure is being prepared for publication.

Results and conclusions: *H. pylori* infection, chronic gastritis, and smoking are all independent predictors of gastric cancer. Higher relative risks were noted with the diffuse type of gastric cancers, whereas much lower risks were noted with intestinal type of gastric cancers, after adjusting for these risk factors (Suzuki et al., *Cancer Epidemiology, Biomarkers and Prevention* 2007; 16:1224–8). 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). Radiation risk was significant only for people without chronic gastritis in developing diffuse type non-cardiac gastric cancers.

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), Akahoshi M (CN), Suzuki G, Nishi N, 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. Study subjects total 224 HCC cases and 644 non-HCC controls for whom stored sera are available. Three controls were selected 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 RR of HCC for liver dose of radiation. After adjusting for alcohol consumption, smoking, and BMI, the RR at 1 Gy of radiation exposure for HCC was 1.67 (P = 0.003), while the RRs for HBV or HCV infection were 63 (P < 0.001) and 83 (P < 0.001), respectively. These estimates changed little when radiation and viral effects were fit jointly. The RR at 1 Gy of radiation exposure for non-B, non-C HCC was 2.74 (P = 0.007) with adjustment for alcohol consumption, smoking, and 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, smoking, and BMI. Results have been published (Ohishi et al., *Hepatology* 2011, in press).

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 published in *Radiation Research*. In 2010, a research protocol was approved to develop appropriate analytical methods for the joint relationship of radiation and hormones upon cancer risk. Data will be analyzed using the developed analytical methods. A manuscript regarding association between ferritin and breast cancer has been submitted to a journal.

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, 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 to 1996. 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),

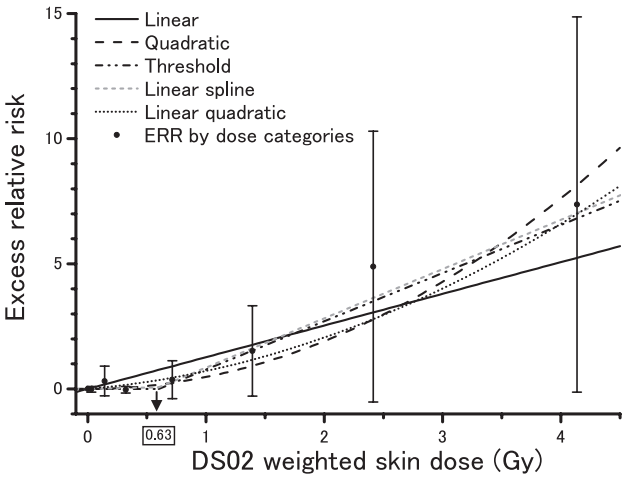

Figure. Risk of incidence of basal cell carcinoma of skin by dose in various models for dose-response relationship. The models include sex, period at diagnosis, log attained age, and AHS membership as background parameters, and age at exposure as an effect modifier. Closed circles are point estimates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

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 was a linear threshold model with a dose threshold at 0.63 Gy (95% CI = 0.34–0.89) estimated by likelihood profile method. This model predicted an ERR/Gy and ERR at 1 Gy of 2.0 (95% CI = 0.69–4.3) and 0.74 (95% CI = 0.26–1.6), respectively. 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 0.63 Gy, lower than the 1.0 Gy reported in the previous study. A manuscript is in preparation and will be submitted in 2011.

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 476 cases of ML, including 306 cases of B-cell origin (140 cases of diffuse large B-cell lymphoma), 130 cases of T-cell origin, and 13 cases of Hodgkin's lymphoma. Presently, characteristics of cases by individual dose are being reviewed and radiation risk assessed.

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.

Dr. Furukawa's paper on the joint effects of smoking and radiation on lung cancer was published (*Radiation Research* 2010; 174:72–82). 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.

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. Presently, an addendum to the RP is being prepared to extend the follow-up period through 2005 based on local cancer and tissue registry data.

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 (RPs 5-08 and 6-10), 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). A paper on lung cancer has been published in *Radiation Research* (Furukawa et al., 2010; 174:72–82). 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), Taga M (R), Imai K (R), Nakachi K, Kusunoki Y (R)

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.

The risk estimation of myelodysplastic syndrome (MDS) conducted in Nagasaki showed a significant linear dose response. The excess relative risk (ERR) at 1 Gy was 4.27 (95% CI, 1.63–9.48). These results have been published (Iwanaga M et al., *Journal of Clinical Oncology* 2011; 29:428–34).

Comprehensive analyses based on the totality of data between 1950 and 2001 are being conducted in collaboration with the Department of Statistics and a manuscript is being prepared.

RP-A5-10 Methods for assessing joint effects of radiation and intermediate risk factors in nested case-control studies

Cologne JB (S), Furukawa K (S), Langholz B, Izumi S, Fujii Y, Hsu WL (S), Grant EJ (EH), Kopecky KJ, Ohishi W (CH), Neriishi K (CH), Fujiwara S (CH), Nakachi K, Cullings HM (S)

Objectives: To develop and evaluate methods for analyzing complex causal models—including mediation (effects of intermediate or intervening variables)—in counter-matched

(exposure-based control selection) nested case-control data.
Background: Analyses of radiation risk and transportation of risk estimates to populations other than the atomic-bomb survivors require consideration of risk factors other than radiation. Some of these factors are radiation related—so-called intermediate risk factors, or mediators—and as such require special methods of analysis. Methods for modeling intermediate risk factors with cohort data, such as structural equations models, are not immediately applicable to data from nested case-control studies. More traditional methods—such as path analysis—may be applied, but do not yield valid estimates of risk.
Design and methods: We will attempt to implement several approaches: path analysis, a regression substitution method, and a full likelihood method. After developing and successfully implementing the methods, we will compare results and assess statistical efficiency via simulation. The first step in evaluation of the methods will be to compare them to standard methods of analyzing complex causal models with cohort data, where the nested case-control sampling design is not a factor.
Subjects and data: The subjects of this research are those already defined under existing RERF RPs. The proposed research involves no new data collection. In terms of personal information protection, RERF procedures for data sharing (collaborative study involving the use of available data) and data-masking procedures will be adhered to.
Time line and expected publications: The research is expected to require about two years. The first year will be devoted to development of the methods and comparison among methods using cohort data. The second year will be devoted to focusing on the likelihood approach and comparing the methods using nested case-control data. Publications produced during the first year should include at least one methodological paper as well as finalization of the AHS liver-cancer cohort study analysis. Publications produced during the second year should include at least one methodological paper, one applied paper on methodological comparisons, and at least two papers based on analyses of joint effects of radiation and intermediate risk factors in the AHS nested case-control studies of breast cancer and liver cancer.
Estimated costs: Expenses related to international travel for RERF researchers and outside collaborators, the frequency, timing, and duration of which will be determined later as the research unfolds. No other expenses are envisioned.

RP-A3-10 A proposal to join the Asia Cohort Consortium. Project 1: Tobacco smoking, alcohol drinking, body mass index, and risk of rare cancers

Grant EJ (EH), Ozasa K (EH), Suyama A (EN), Fujiwara S (CH), Akahoshi M (CN), Shore RE (D)

This proposal outlines the framework by which RERF would participate in the Asian Cohort Consortium (ACC). Additionally, a proposal for the first collaborative project with the ACC is described.

The ACC is a collaborative cohort created in 2008 by researchers in Asia and the U.S.A. The ACC combines Asian cohorts from many institutions to study the relationships between environmental exposures and the etiology of disease (among other goals) with high statistical power. As RERF manages one of the largest and longest-running cohort studies in the world, RERF data would be a valuable addition to the ACC. As an ACC contributor, RERF could participate in ACC manuscripts, which are likely to be high profile publications. RERF would also be able to propose ACC research projects that utilize the statistical power of the combined cohort.

RERF would supply lifestyle information collected from AHS and LSS questionnaires along with cancer incidence data for the current project. Additional projects would require an addendum to the initial RP and may require some additional data (e.g., death due to a particular disease). Data sent to the ACC would be individual data, suitably anonymized to ensure privacy. To avoid biasing results due to ionizing exposure, data would be restricted to cohort members with less than 100 mGy kerma exposures. Released data would be sent to the ACC data coordinating center wherein it would be analyzed by Center statisticians in response to written requests. Data cannot be released to other researchers for analyses nor can any project occur with RERF data without RERF's explicit authorization. An RERF representative would be expected to attend ACC meetings twice per year.

The ACC is currently inviting RERF to participate in a proposed project entitled "Tobacco smoking, alcohol drinking, body mass index, and risk of rare cancers." Rare cancers would include cancers of the salivary glands, small intestine, adrenal gland, and male breast. As the background rates of these cancers are very low, the associations of these cancers with lifestyle factors are unknown. By pooling cohort data, important insights may be generated regarding the etiology of these cancers.

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 U.S. National Cancer Institute where the pooled data are being analyzed. The first step of this collaborative study—analysis of thyroid cancer after radiotherapy for childhood cancer—has been completed, and a dataset including RERF's data for the analysis in the next step is being prepared.

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 2011.

Special Cancer Studies Publications

RERF Reports (RR)

♦ 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 2010 (July); 174(1):72–82.

[Abstract] While radiation increases the risk of lung cancer among members of the Life Span Study (LSS) cohort of atomic bomb survivors, there are still important questions about the nature of its interaction with smoking, the predominant cause of lung cancer. Among 105,404 LSS subjects, 1,803 primary lung cancer incident cases were identified for the period 1958–1999. Individual smoking history information and the latest radiation dose estimates were used to investigate the joint effects of radiation and smoking on lung cancer rates using Poisson grouped survival regression methods. Relative to never-smokers, lung cancer risks increased with the amount and duration of smoking and decreased with time since quitting smoking at any level of radiation exposure. Models assuming generalized interactions of smoking and radiation fit markedly better than simple additive or multiplicative interaction models. The joint effect appeared to be super-multiplicative for light/moderate smokers, with a rapid increase in excess risk with smoking intensity up to about 10 cigarettes per day, but additive or subadditive for heavy smokers smoking a pack or more per day, with little indication of any radiation-associated excess risk. The gender-averaged excess relative risk per Gy of lung cancer (at age 70 after radiation exposure at 30) was estimated as 0.59 (95% confidence interval: 0.31–1.00) for nonsmokers with a female:male ratio of 3.1. About one-third of the lung cancer cases in this cohort were estimated to be attributable to smoking while about 7% were associated with radiation. The joint effect of smoking and radiation on lung cancer in the LSS is dependent on smoking intensity and is best described by the generalized interaction model rather than a simple additive or multiplicative model.

♦ 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 2010 (April); 116(7):1646–55.

[Abstract] ***Background:*** Radiation exposure is an established cause of clinical thyroid cancer, but little is known about radiation effects on papillary microcarcinoma (PMC) of the thyroid, a relatively common subclinical thyroid malignancy. Because the incidence of these small thyroid cancers has been increasing, it is important to better understand them and their relation to radiation.

Methods: PMCs were identified in a subset of 7,659 members of the Life Span Study of atomic bomb survivors who had archived autopsy or surgical materials. We conducted a pathology review of these specimens and evaluated the histological features of the tumors and the association between PMCs and thyroid radiation dose.

Results: From 1958 to 1995, 458 PMCs were detected among 313 study subjects. The majority of cancers exhibited pathologic features of papillary thyroid cancers. Overall, 81% of the PMCs were of the sclerosing variant and 91% were nonencapsulated, psammoma bodies that occurred in 13% and calcification was observed in 23%. Over 95% had papillary or papillary-follicular architecture

and most displayed nuclear overlap, clear nuclei, and nuclear grooves. Several of these features increased with increasing tumor size, but no association was found with radiation dose. A significant radiation-dose response was found for the prevalence of PMCs (estimated excess odds ratio/Gy = 0.57; 95% confidence interval, 0.01–1.55), with the excess risk observed primarily among women. ***Conclusion:*** Exposure to low-to-moderate doses of ionizing radiation appears to increase the risk of thyroid PMCs, even when exposure occurs during adulthood.

♦ Iwanaga M, Hsu WL, Soda M, Takasaki Y, Tawara M, Joh T, Amenomori T, Yamamura M, Yoshida Y, Koba T, Miyazaki Y, Matsuo T, Preston DL, Suyama A, Kodama K, Tomonaga M: Risk of myelodysplastic syndromes in people exposed to ionizing radiation: A retrospective cohort study of Nagasaki atomic bomb survivors. Journal of Clinical Oncology 2011 (February); 29(4):428–34.

 (RR 14-09)

[Abstract] ***Purpose:*** The risk of myelodysplastic syndromes (MDS) has not been fully investigated among people exposed to ionizing radiation. We investigate MDS risk and radiation dose-response in Japanese atomic bomb survivors. ***Patients and methods:*** We conducted a retrospective cohort study by using two databases of Nagasaki atomic bomb survivors: 64,026 people with known exposure distance in the database of Nagasaki University Atomic-Bomb Disease Institute (ABDI) and 22,245 people with estimated radiation dose in the Radiation Effects Research Foundation Life Span Study (LSS). Patients with MDS diagnosed from 1985 to 2004 were identified by record linkage between the cohorts and the Nagasaki Prefecture Cancer Registry. Cox and Poisson regression models were used to estimate relationships between exposure distance or dose and MDS risk. ***Results:*** There were 151 patients with MDS in the ABDI cohort and 47 patients with MDS in the LSS cohort. MDS rate increased inversely with exposure distance, with an excess relative risk (ERR) decay per km of 1.2 (95% CI, 0.4 to 3.0; P < .001) for ABDI. MDS risk also showed a significant linear response to exposure dose level (P < .001) with an ERR per Gy of 4.3 (95% CI, 1.6 to 9.5; P < .001). After adjustment for sex, attained age, and birth year, the MDS risk was significantly greater in those exposed when young. ***Conclusion:*** A significant linear radiation dose-response for MDS exists in atomic bomb survivors 40 to 60 years after radiation exposure. Clinicians should perform careful long-term follow-up of irradiated people to detect MDS as early as possible.

Other Journal Publication

♦ Ohishi W, Chayama K: Hepatitis C virus RNA and nucleic acid testing. Nippon Rinsho [Japanese Journal of Clinical Medicine] 2010 (June); 68(Suppl 6):450–3. (Japanese)

Manuscripts in Press

⌘ Grant EJ, Neriishi K, Cologne JB, Eguchi H, Hayashi T, Geyer SM, Izumi S, Nishi N, Land CE, Stevens RG, Sharp GB, Nakachi K: Association of ionizing radiation and breast cancer-related serum hormone and growth factor levels in cancer-free female A-bomb survivors. Radiation Research.

⌘ Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Nishi N, Tsuge M, Chayama K: Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. Hepatology.

Special Cancer Studies Oral Presentations

❖ Furukawa K, Preston DL, Funamoto S, Egawa H, Tokuoka S, Ozasa K, Mabuchi K. Radiation and smoking effects on lung cancer incidence by histological types among atomic-bomb survivors. 2010 ASA (American Statistical Association) Conference on Radiation and Health, 13–16 June 2010, Annapolis, Maryland, USA

❖ Misumi M, Sugiyama H, Kishikawa M, Iseki M, Yonehara S, Hayashi T, Soda M, Tokuoka S, Sakata R, Grant EJ, Ron E, Mabuchi K, Kasagi F, Suyama A, Ozasa K. Radiation risk of skin cancer among RERF Life Span Study cohort (1958–1996). 2010 ASA (American Statistical Association) Conference on Radiation and Health, 13–16 June 2010, Annapolis, Maryland, USA

❖ Cologne JB, Grant EJ, Berrington A, Neriishi K, Nakachi K. Joint effects of radiation and serum estradiol on pre- and post-menopausal breast cancer risk in female atomic-bomb survivors: A nested case-control study involving an intermediate factor. Scientific Meeting for Cancer Prevention 2010 Sapporo, 15–16 July 2010, Sapporo

❖ Ohishi W, Fujiwara S, Cologne JB, Akahoshi M, Tsuge M, Chayama K. Effects of radiation exposure and lifestyle on risk of non-B, non-C hepatocellular carcionma. The 14th Annual Meeting of the Japan Society of Hepatology, 13–15 October 2010, Yokohama

❖ Iwanaga M. Epidemiology and clinical characteristics of leukemia and myelodysplastic syndromes (MDS) in atomic bomb survivors. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Tsuge M, Chayama K. Effects of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. The 61st Annual Meeting of the American Association for the Study of Liver Diseases, 29 October–2 November 2010, Boston, Massachusetts, USA

❖ Furukawa K, Taga M, Misumi M, Preston DL, Ozasa K, Mabuchi K. Radiation and lung cancer: Epidemiological and genetic findings from studies among atomic-bomb survivors. The 7th International Conference on High Levels of Natural Radiation and Radon Areas, 24–26 November 2010, Mumbai, India

❖ Iwanaga M, Hsu WL, Soda M, Miyazaki Y, Preston DL, Suyama A, Ozasa K, Kodama K, Tomonaga M. Risk of myelodysplastic syndromes (MDS) in Nagasaki atomic bomb survivors. Nagasaki University Global COE Program "International Symposium on Radiation Epidemiology and Radiobiology," 29–30 November 2010, Nagasaki

Research Protocol 18-61

Tumor and Tissue Registries, Hiroshima and Nagasaki

Note that RERF studies related to the tumor and tissue registries include RPs 6-10, 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, A5-10, A3-10, A12-08, and A5-08 (all discussed under Special Cancer Studies*).*

RP 18-61 Tumor and tissue 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. 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. In addition, tissue registries to collect and archive tumor tissue samples were started in 1973 under the auspices of the Hiroshima Prefectural Medical Association and in 1974 by the Nagasaki City Medical Association. Pathology slides and pathology reports are collected for each tumor, malignant or benign. Having 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. 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. Similarly, the tissue registries were recently placed under the auspices of the respective prefecture governments. The registry information is linked with the master list of the members of RERF's major cohort samples (LSS, *in utero*, and F_1) with the permission of each registry and serves as the source of RERF cancer incidence data.

Case collection by notifications and death certificates is almost completed through 2009 in both Hiroshima and Nagasaki. On-site record abstraction is nearly complete through 2005 in Hiroshima and through 2007 in Nagasaki. As for tissue registry, case collection and registration have been completed through 2007 in both Hiroshima and Nagasaki. The linkage between tumor and tissue registry information and RERF subjects has been completed through 2003.

The Hiroshima and Nagasaki tumor registry data, complemented by tissue registries both qualitatively and quantitatively, have regularly been included in publications and websites 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, as the data satisfy their accuracy standards. 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.

Tumor and Tissue Registries Publications

Journal Publications

♦ 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 2010 (April); 82(4):668–74.

♦ 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] 2010 (April); 63(4):304–6. (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

♦ Utada M, Ohno Y, Hori M, Soda M, Suyama A: Analysis of the standardization and centralization for cancer treatment in Nagasaki Prefecture. Asian Pacific Journal of Cancer Prevention 2010; 11(2):409–12.

Manuscript in Press

⌘ Samartzis D, Nishi N, Hayashi M, Cologne JB, Cullings HM, Kodama K, Miles EF, Funamoto S, Suyama A, Soda M, Kasagi F: Exposure to ionizing radiation and development of bone sarcoma: New insights based on atomic-bomb survivors of Hiroshima and Nagasaki. Journal of Bone and Joint Surgery. American Volume. (related to *Life Span Study*)

Tumor and Tissue Registries Oral Presentations

❖ Suyama A. Nagasaki cancer registry. The 26th Annual Meeting of the Japanese Society of Clinical Cytology, Kyusyu Branch, 5 September 2010, Nagasaki

❖ Kodama K, Ozasa K, Sugiyama H, Soda M, Suyama A, Katayama H, Shore RE. Radiation and cancer incidence in atomic bomb survivors—Effective use of cancer registry data. The 32nd Annual Meeting of the International Association of Cancer Registries, 12–14 October 2010, Yokohama (related to *Adult Health Study*)

❖ Soda M, Suyama A, Sekine I, Furukawa M, Igawa T, Sakai H. Decreased prostate cancer mortality observed in Sasebo City, Nagasaki, Japan, with introduction of PSA screening. The 32nd Annual Meeting of the International Association of Cancer Registries, 12–14 October 2010, Yokohama

❖ Nagayoshi A, Soejima M, Yoshida M, Hayama S, Yamakawa S, Yamada T, Soda M, Suyama A. Cancer registry public relations activities at the open house events of the Radiation Effects Research Foundation. The 19th Annual Meeting on Japanese Association of Cancer Registries, 15 October 2010, Yokohama

❖ Soda M. Nagasaki Prefecture's cancer registry system and its significance (Incidence of prostate cancer in Nagasaki Prefecture). FY2010 Joint Meeting of the Prostate Screening Council Operating Committee and the Anti-Prostate Cancer Promotional Committee, 11 December 2010, Tokyo

❖ Kodama K, Ozasa K, Sugiyama H, Soda M, Suyama A,

Katayama H, Shore RE, Okubo T. Radiation effects on cancer risks in the Life Span Study cohort. The 13th Coordination and Planning Meeting of the WHO REMPAN Collaborating Centres and Liaison Institutions, 16–18 February 2011, Nagasaki (related to *Adult Health Study*)

Research Protocols 3-04, 1-92, 10-86, 18-59, A4-10
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. Thus far, a total of 26 molars were measured. As the total number is so small, it is too early to evaluate deviations of individual doses from DS02 estimates. We asked Nagasaki University to collect tooth samples of A-bomb survivors as a part of their Global Center of Excellence (COE) program and they agreed. Their ethics committee approved the collection of teeth in September 2010.

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. We evaluated individual doses from A-bomb survivors in Hiroshima who were present at distances of about 3 km or further from the hypocenter, and have DS02 estimated doses of less than 5 mGy. A total of 56 molars donated by 49 AHS participants were examined. They were ≥10 years old at the time of bombing and had no record of radiotherapy. Individual doses were estimated by measuring CO_2^- radicals in tooth enamel with the electron spin resonance (ESR; or electron paramagnetic resonance, EPR) method. The results provided their estimated doses which varied from –200 mGy to 500 mGy. The median dose was 17 mGy for the buccal parts and 13 mGy for the lingual parts of the molars. Three molars had ESR-estimated doses of 300 to 400 mGy for both parts, which indicates possible exposures to excess doses of penetrating radiation, although the origin of such radiation remains to be determined. The results did not support claims that a large fraction of distally-exposed survivors received large doses (e.g., 1 Gy) of external penetrating radiation resulting from residual radiation.

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 2011, we have collected 1,505 tooth samples from Hiroshima AHS participants and 50 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	305 (11)*	15 (0)
1–30	366 (2)	0 (0)
31–100	317 (4)	5 (0)
101 and over	270 (3)	14 (1)
Not available	247 (3)	16 (0)
	Total 1,505 (23)	Total 50 (1)

* Numbers in parentheses represent cases collected during April 2010–March 2011.

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 organ-specific 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 ~28,700 (~22,000 in the LSS) 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. RERF received a system from a contractor that will allow this type of improved transformation of U.S. Army map coordinates to new coordinates. In addition, workers in the Master File Section, Department of Epidemiology, are actively engaged in locating survivor shielding history neighborhood diagrams on the photographic maps.

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, U.S.A.) 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, U.S.A.) 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.

RP-A4-10 Semi-parametric methods using radiation exposure and chromosome aberration data in atomic-bomb survivor studies

Wang CY, Cullings HM (S), Song X, Ozasa K (EH), Soda M (EN), Suyama A (EN), Kodama Y (G), Davis S, Kopecky KJ

This research will develop semi-parametric statistical methods to adjust for the effects of radiation dose measurement error on the estimation of radiation dose responses for health effects in survivors of the atomic bombings of Hiroshima and Nagasaki. Although some measurement error methods have been applied to adjust for radiation measurement error in RERF data, further development of semi-parametric or non-parametric methods

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.

is important to understand the radiation effect to cancer or other outcome variables. Dosimetry data may be considered as a surrogate variable for the unobserved underlying radiation exposure. Biomarkers such as percentage of stable chromosome aberrations can be treated as a type of instrumental variable for the unobserved radiation dose. An important aspect of this work is that the measurement error standard deviation will not have an assumed value, but rather will be estimated from the data, even though the data do not include replicate measurements or estimates of radiation doses. This RP was approved in November 2010 and the paperwork for data sharing is presently being completed.

Atomic-bomb Dosimetry Studies Publications

Commentary and Review Series (CR)

♦ Cullings HM, Smith KR: Better radiation exposure estimation for the Japanese atomic-bomb survivors enables us to better protect people from radiation today. Journal of Exposure Science and Environmental Epidemiology 2010 (November); 20(7):575–6. (CR 1-10)

[Abstract] The development of radiation dose estimates for the Japanese atomic-bomb survivors illustrates the value of exposure science in greatly increasing the precision of risk estimates and, as a result, global confidence in the details of the health impacts from ionizing radiation and the development of health-protective regulations and guidance.

Other Journal Publications

♦ Cullings HM: A preliminary geospatial analysis of ^{137}Cs measured in soil cores from Hiroshima. Research Group on the Black-rain Fallout of the Hiroshima A-bomb, ed. Current Status of Studies on Radioactive Fallout with "Black-rain" Due to the Hiroshima Atomic Bomb. 2010 (April), pp 119–33.

♦ 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] 2010 (April); 63(4):267–9. (Proceedings of the 50th Late A-bomb Effects Research 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] 2010 (April); 63(4):261–4. (Proceedings of the 50th Late A-bomb Effects Research Meeting, 2009) (Japanese)

Atomic-bomb Dosimetry Studies Oral Presentations

❖ Cullings HM. Uncertainty in dose reconstruction for the atomic bomb survivors in Hiroshima and Nagasaki. The 55th Annual Meeting of the Health Physics Society, 27 June–1 July 2010, Salt Lake City, Utah, USA

❖ Cullings HM, Kerr GD, Egbert SD, Funamoto S. Energy spectra of the shielded and organ fluences of neutrons and gamma rays calculated by dosimetry system DS02 for atomic bomb survivors in Hiroshima and Nagasaki. The 55th Annual Meeting of the Health Physics Society, 27 June–1 July 2010, Salt Lake City, Utah, USA

❖ Egbert SD, Kerr GD, Cullings HM, Funamoto S. Neutron and gamma-ray free-in-air spectra at Hiroshima and Nagasaki increases in energy with range and city resulting in decreasing effectiveness. The 55th Annual Meeting of the Health Physics Society, 27 June–1 July 2010, Salt Lake City, Utah, USA

❖ Nakamura N, Hirai Y. Evaluation of individual doses of atomic-bomb survivors by ESR using tooth enamel. The 14th ESR Forum Research Meeting, 16 July 2010, Yamaguchi

❖ Hirai Y, Kodama Y, Cullings HM, Nakamura N. Electron spin resonance analysis of tooth enamel provides no evidence for exposures to large radiation doses in distally exposed A-bomb survivors. The 35th Annual Meeting of the Chugoku Area Radiation Research Society, 26 July 2010, Hiroshima

❖ Cullings HM. Geospatial analysis of ^{137}Cs levels measured in Hiroshima soil samples collected in 1976 and 1978. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Hirai Y, Kodama Y, Cullings HM, Nakamura N. ESR dose estimation using tooth enamel from Hiroshima A-bomb survivors: VI. ESR gamma dose in distally exposed A-bomb survivors. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto

❖ Rühm W, Wallner A, Cullings HM, Egbert SD, El-Faramawy N, Faestermann T, Kaul DC, Knie K, Korschinek G, Nakamura N, Roberts J, Rugel G. Estimation of neutron dose of atomic bomb survivors by means of accelerated mass spectrometry measurement of ^{41}Ca/Ca ratio in tooth enamel. The 53rd Annual Meeting of the Japan Radiation Research Society, 20–22 October 2010, Kyoto