

Active Research Protocols by Study Program

1 April 2012–31 March 2013

The 110 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 Associate 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, 1-75 (Platform Protocol), 2-61, A2-11, A1-11, A2-10, A1-09, A11-08, 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), Kodama K (CS), Peto R

Objectives: Estimation of the effects of smoking on mortality among the LSS population.

Background and Significance: It has been suggested that the effects of smoking on mortality in the Japanese population are smaller than elsewhere. It is not clear whether this is a reflection of the fact that cigarette smoking became common in Japan later than in other countries, or there are other risk-reducing factors, or the observation is an artifact of using different methodologies to estimate the smoking risk.

Study Methods: LSS individuals contributed to person-years at risk from the earliest of 1 January 1965 or one year following the first survey at which they provided information on smoking status. Follow-up was to 1 January 2008. When new smoking information was obtained via a subsequent survey, the person was reclassified and contributed to the person-years at risk in the updated category one year later. Age-standardized death rates by sex, attained age, calendar year, and smoking status were calculated using Poisson regression. Age-standardized death rates in each birth decade were obtained as a weighted average of the fitted rates at each level of age, with weights equal to the overall proportion of the total person-years in each age category. An analysis taking into account radiation dose and other potential confounding variables was also carried out.

Study Progress: A paper was published (Sakata R et al., *BMJ* 2012; 345:e7093).

Results and Conclusions: Smokers born in later decades tended to smoke more cigarettes per day than those born earlier, and to have started smoking when younger. Among smokers born during 1920–45 and who started smoking before age 20, the overall mortality was more than doubled in both sexes (rate ratios versus never smokers: males 2.21 [95% confidence interval 1.97 to 2.48], females 2.61 [1.98 to 3.44]) and life expectancy was reduced by about a decade (8 years for males, 10 years for females). Those who stopped smoking before age 35 avoided all of the excess risk among continuing smokers, while those who stopped smoking before age 45 avoided most of it.

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

Sakata R (EH), Nishi N, Nagano J, Grant EJ (EH), Sugiyama H (EH), Ohishi W (CH), Akahoshi M (CN), Moriwaki H (EH), Mabuchi K, Ozasa K (EH), Kodama K (CS)

Objectives: To update the information on epidemiological factors that may confound or modify radiation effects among LSS subjects, and to obtain information on subsequent medical radiation exposures.

Background and Significance: Increased rates of malignant tumors and circulatory diseases indicate that important late effects of A-bomb radiation on health include common multifactorial diseases. Therefore, it is necessary to obtain as much information on various environmental, lifestyle, and endogenous factors as possible to properly evaluate the health effects of A-bomb radiation. ABCC-RERF has conducted several mail surveys for this purpose in the past. Since more than 15 years had passed since the last mail survey, another survey was conducted to update information on factors that might have changed longitudinally and to obtain new information on factors that need clarification from previous studies. For the first time, the survey collected information on exposures from radiotherapy and relatively high-dose diagnostic radiation procedures (e.g., CT scans).

Study Methods: Although the original targets of the survey were all 47,000 subjects who were alive as of 1 July 2007 in the Extended Life Span Study cohort (LSS-E85), the number was reduced to about 25,000 because of a new legal restriction regarding access to the resident registries, which limited our ability to obtain addresses. The content, validity, reproducibility, and feasibility of the questionnaire were evaluated through a pilot study (B45-06) as well as outside review.

Study Progress: The survey was sent to 24,640 subjects. Data entry of received answers was finished in February 2012 and data cleaning is ongoing at present. A summary report was written and sent to the subjects who returned a questionnaire.

Results and Conclusions: The number of returned questionnaires with answers was 14,090. The response rate for males was slightly higher than that for females (60.4% for male, 55.3% for female). Sixty percent of respondents were female. Respondents in their seventies were most prevalent among males (60's 37%, 70's 42%, over 80 years 21%), while the majority of females were over 80 years (60's 27%, 70's 32%, over 80 years 41%).

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

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

Objectives: To ascertain the long-term health effects of exposure to ionizing radiation.

Background and Significance: Evaluation of the late health effects of radiation from the atomic bombs is the central goal of RERF research. It can best be accomplished by careful and complete follow-up of the health of the survivors in the Life Span Study (LSS).

Study Methods: A cohort study. The LSS cohort of 120,000 subjects has been followed up since 1950 and individual A-bomb radiation doses have been estimated. They have been followed up for their vital status, cause of death, and cancer incidence. Mail surveys have been conducted to obtain information on factors that might confound or modify the radiation effects.

Study Progress: Analyses of updated cancer and noncancer mortality data (through 2003) using the DS02 dosimetry were published in *Radiation Research*. Analysis of mortality of non-cancer respiratory and digestive disease is being conducted in RP-A1-11. Analyses of the effects of co-morbidity from other chronic diseases on the mortality of major non-cancer diseases are being conducted in RP-A2-11. Analysis of updated cancer incidence data (through 2005) has been initiated. Estimation of the LSS population in the cancer registry areas in Hiroshima and Nagasaki is being updated in collaboration with the Department of Statistics. In coordination with the RERF Dosimetry Committee, effects of fallout rain based on the information from early surveys and other available sources are being investigated.

Results and Conclusions: An increased risk of solid cancer with radiation dose appears to persist throughout life. The risks also apparently increased with radiation dose for some types of noncancer disease, but whether those associations might be spurious is carefully being investigated by examining disease misclassification (e.g., underlying malignancies) and possible confounding. Since a majority of the subjects who were exposed when young are still alive and risk estimates for them are uncertain at this stage, it is essential to continue their follow-up.

RP 2-61 Study of mortality and cancer incidence in people exposed *in utero*

Sugiyama H (EH), Shimizu Y (EH), Preston DL, Suyama A, Cologne JB (S), Misumi M (S), Ozasa K (EH), Kodama K (CS)

Objectives: To investigate the nature of radiation effects on mortality and cancer incidence among people exposed *in utero* to atomic-bomb radiation.

Background and Significance: Prenatal medical diagnostic X-ray exposure has been associated with increased risk of childhood cancer in several case-control studies. The RERF *in utero* cohort, although small in size, has a large collective dose, with a subgroup with substantial radiation doses, and so can provide much information on the sensitivity of the embryo and fetus to late radiation effects. It is the only available cohort in the world with exclusively *in utero* radiation exposure and adulthood data on health risks.

Study Methods: Follow-up of mortality and cancer incidence in a cohort of 3,600 people who were *in utero* at the time of the bombings has now continued to age 62.

Study Progress: Analysis of the mortality data for 1950–2008 is underway with special emphases on the magnitudes and temporal patterns of radiation risks for cancer and noncancer deaths.

Results and Conclusions: Significant associations between radiation exposure and solid cancer mortality (ERR/Gy = 1.2; 95% CI: 0.11, 3.2), and noncancer disease mortality (ERR/Gy = 1.4; 95% CI: 0.36, 3.2) among *in utero* survivors were detected.

RP-A2-11 Mortality analysis of Life Span Study (LSS) cohort taking into account multiple causes of death indicated in death certificates

Takamori A, Kasagi F, Takahashi I (CH, EH), Ozasa K (EH), Yanagawa T

Objectives: To study the association between radiation and

underlying/secondary causes of death that were identified from death certificates of LSS subjects.

Background and Significance: The LSS has mainly focused on relationship between underlying causes of death and radiation dose. However, diseases other than underlying causes of death (secondary causes of death) may modify the radiation risk estimates of death compared to the underlying cause of death and have rarely been studied.

Study Methods: The LSS follow-up period considered was 1950–2002. We are investigating whether the dose response determined only by underlying causes of death is different from that determined by both the underlying and secondary causes of death.

Study Progress: The dataset was established and is being analyzed.

Results and Conclusions: During all follow-up, a total of 49,603 deaths were identified among 86,611 LSS subjects. Circulatory disease was identified as the underlying cause of death in 18,705 deaths. Respiratory disease was identified as one of the secondary causes of death in 2,020 deaths, renal disease in 498 deaths, diabetes mellitus in 401 deaths, and any cancer in 224 deaths.

RP-A1-11 Radiation exposure and the risk of mortality from noncancer diseases of the respiratory and digestive systems in the Life Span Study (LSS), 1950–2005

Pham TM, Sakata R (EH), Grant EJ (EH), Shimizu Y (EH), Furukawa K (S), Takahashi I (CH, EH), Sugiyama H (EH), Soda M (EN), Ozasa K (EH)

Objectives: To examine the association between atomic-bomb radiation and the mortality risk of noncancer diseases of the respiratory and digestive systems in the LSS, 1950–2005. The goal is to examine the main specific conditions within those broad classifications and to determine the degree to which the apparent associations with radiation may be due to misdiagnoses of underlying cancer or cardiovascular disease.

Background and Significance: Findings regarding an association of radiation exposure and noncancer respiratory and digestive diseases have been sparse and non-conclusive. Previously examined radiation risks for those diseases in the LSS were derived from aggregate conditions.

Study Methods: The follow-up data from 1950 to 2005 for 86,611 subjects of the LSS cohort are being analyzed. The estimated risk will be adjusted for potential confounding variables (smoking, alcohol, body mass index, education, diabetes, occupation, and proximal/distal survivors) and for incidence/co-morbidity of cancer and co-morbidity of cardiovascular disease. Period-specific analyses will also be conducted since background disease structure in Japan has changed markedly in these several decades.

Study Progress: For respiratory disease, pneumonia/influenza was predominant and likely to drive the whole association. No risks at low exposure levels were observed, but a linear dose-response relationship was observed in the late period of 1980–2005. Adjustment for cancer and for cardiovascular diseases reduced the radiation risk by about 35%. A draft on respiratory diseases has been completed and submitted to the internal review. For digestive diseases, the linear excess relative risk estimates were small for the whole

period. Liver disease accounted for approximately half of all digestive disease deaths. Some increased radiation risk was observed in the middle follow-up period, while the risks in the early as well as in the late periods tended to be small. In an effort to assess the impact of misdiagnoses due to co-morbidity, we censored subjects at the time of cancer diagnosis. Censoring reduced the radiation risks for all disease subcategories but the pneumonia/influenza subcategory remained significantly elevated. However, uncertainties remain because of limitations of the co-morbidity data.

Results and Conclusions: Although the biological mechanisms by which radiation risks were elevated remain unclear, the difference in risks by period of follow-up may provide clues regarding confounding of the associations.

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, Soda M (EN), Suyama A, Ozasa K (EH), Cullings HM (S)

Objectives: To examine the robustness of a semi-parametric survival extrapolation method based on the constant excess hazard model (developed by Dr. Hwang) using LSS cohort data. The long follow-up data in the LSS cohort provide a good opportunity to evaluate temporal extrapolation using the model.

Background and Significance: The knowledge of how long patients can expect to live after the diagnosis of a disease is essential for cost-effectiveness evaluation of medical interventions. Researchers need to look beyond the follow-up limit of clinical trials and take a lifetime perspective when measuring the impact of a new medical therapy. Because there are usually only very limited follow-up data for newer medical interventions, a robust statistical method for survival extrapolation is particularly important. Previously-used parametric survival models can make reasonably good short-term prediction. Problems may arise, however, when there is a high right-censoring rate or long-term projection.

Our group therefore conceived an innovative approach that borrows information from national vital statistics data, and incorporates this information in a semi-parametric survival extrapolation method based on the logit survival ratio between patients with a disease and an age- and gender-matched reference population (see details in the Methods section), assuming a constant excess hazard. In that case, the logit survival ratio curve will converge to a straight line over time, which allows linear extrapolation.

Study Methods: We propose to examine whether the logit survival ratio curves between atomic-bomb survivors who (1) are with or without radiation exposure, or (2) had a specific cancer vs. an age- and gender-matched reference population generated from Japanese vital statistics by a Monte Carlo method, will converge to a straight line over time. In addition, we will test different strategies for estimating the slope in logit survival ratio plots in order to find the best strategy for choosing the sample size. A software program has been developed by Prof. J-S Hwang (Academia Sinica, Taiwan) to facilitate these calculations.

Study Progress: Statistical analysis has been completed.

Results and Conclusions: For the period 1950–1998, the logit survival ratio between atomic-bomb survivors who were exposed to the highest-dose radiation (HDR) (dose $\geq 1,000$ mGy) (2,375 persons) and an age- and gender-matched reference group selected from those without radiation exposure (17,830 persons), the slope eventually randomly fluctuated between +0.005 and -0.020 . For site-specific cancers (stomach, lung, liver, colon, breast, pancreas) the logit survival ratio between patients with cancer and the matched references, and the slopes also show overt random variations over time as the sample size decreases, especially for cancers with a smaller sample size. To solve this problem, we derived two new mathematical theorems on the properties of the logit survival ratio, based on the constant excess hazard assumption. Using these, we developed a set of simple yet robust rules for selecting the best slope for extrapolating the survival curves. With the selected slope, we further evaluate the accuracy of long-term projection by comparing the difference between the predicted and actual survival curves. The semi-parametric model had excellent accuracy in extrapolation: the differences between the predicted and actual survival probabilities at the end of follow-up were all below 0.05 (in absolute value). We concluded that the semi-parametric method based on the constant excess hazard assumption is a robust statistical method for lifetime survival extrapolation.

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, Suyama A

Objectives: Apply a biologically based two-mutation carcinogenesis model to individual leukemia incidence data in the LSS cohort.

Background and Significance: The Netherlands National Institute for Public Health and the Environment (RIVM) has a history of developing Moolgavkar-type two-mutation models for leukemia in laboratory animals and humans that attempt to incorporate biological concepts of the disease. The biologically-based leukemia model results can be compared with our empirical-model results, and may be informative as to how to transfer risk estimates to other radiation-exposed groups, such as western populations with chronic or low-dose exposures.

Study Methods: RIVM has developed a maximum-likelihood, Two Mutation Carcinogenesis (TMC) model that takes relevant radiobiological information into account more than most other two-stage models have done by estimating mutation rates of interest. Risks will be estimated for LSS data for three main radiogenic leukemia subtypes (acute myeloid leukemia, chronic myeloid leukemia, and acute lymphocytic leukemia).

Study Progress: An earlier analysis under the assumption of a fixed lag time led to problems. A minimum lag time between the creation of the first malignant cell and leukemia diagnosis provides better model fitting. The value for lag time obtained in statistical data fitting is largely determined by the earliest cases. Since the LSS does not contain information on cases that occurred in the first years after the bombings, using the LSS data alone results in implausibly large values. In the past year, the limited information

available on early cases was used to obtain a more realistic value for the lag time. Data fitting to determine the sensitivity of the other parameters in the model to the uncertainty in the lag time are in progress.

Results and Conclusions: Preliminary TMC analyses indicated that different background mutation rates were found for men and women. All other parameters are identical for the two sexes. This suggests that risk estimates for populations with different baseline leukemia incidence can be obtained from the model. Similarly, risks can be derived for chronic exposures. This work was presented at the 14th International Congress of Radiation Research (ICRR), Warsaw, Poland, 2011.

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, Suyama A, Sakata R (EH), Akahoshi M (CN)

Objectives: The purpose of this study is to evaluate whether 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 the Life Span Study (LSS) cohort members. Specifically, it will:

- 1) Evaluate whether radiation dose without adjustment for co-factors is associated with cause-specific kidney disease mortality in the LSS cohort,
- 2) 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,
- 3) 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,
- 4) Evaluate the validity of self-report 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 Adult Health Study (AHS).

Background and Significance: Multiple studies of those exposed to whole body and chest irradiation have demonstrated that they are at increased risk of fatal cardiovascular disease in relation to the cumulative dose-volume of radiation exposure to the heart. Recent work at RERF has also demonstrated that radiation dose is associated with risk of hypertensive heart disease and related to systolic and diastolic blood pressure. This suggests the possibility that the risk of cardiovascular disease (CVD) in atomic-bomb survivors (and thus others exposed to whole body radiation) may be mediated in part by damage to the kidney, because the kidney is a key organ involved with blood pressure regulation, and hypertension is a well-known risk factor for myocardial infarction.

Study Methods: This study used a retrospective cohort design using the LSS as the population base restricted to those with known radiation doses that were in the cities at the time of bombing. As no clinical screening was done as part of the LSS, hypertension and diabetes mellitus were obtained by self-report from LSS mail surveys. Kidney

disease was obtained from the coding of the causes of death from the death certificate. We analyzed chronic kidney disease as the underlying cause of death and also as a contributing cause of death. The validity of self-report of hypertension and diabetes in the LSS was examined by comparing LSS-questionnaire self-reports with actual clinical findings from the AHS for those where both were available.

Study Progress: All analyses have been completed and a peer-reviewed manuscript has been published.

Results and Conclusions: 86,609 persons with 3.3 million person-years of follow-up were analyzed. We found a significant association between radiation dose and our broadest definition of chronic renal failure (CRF) among the full cohort. A quadratic dose excess relative risk model ($ERR/Gy^2 = 0.091$; 95% CI: 0.05, 0.198) fit slightly better than a linear model. Among those who answered a lifestyle questionnaire, an association was also observed with the broadest CRF definition ($ERR/Gy^2 = 0.15$; 95% CI: 0.02, 0.32). Adjustment for hypertension and diabetes improved model fit but did not substantially change the ERR/Gy^2 estimate, which was 0.17 (95% CI: 0.04, 0.35). We found 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 the incidence of hypertension in this population. Our results suggest that renal dysfunction could be part of the mechanism that causes an increased risk of CVD after whole body irradiation, a hypothesis that deserves further study.

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

Grant EJ (EH), Shimizu Y (EH), Soda M (EN), Sugiyama H (EH), Sakata R (EH), Yamada M (CH), Kubo T, De Roos A, Kopecky KJ, Davis S

Objectives: To evaluate the radiation risk of urothelial cancers (UC) after accounting for lifestyle factors also known to be associated with these cancers. UC is the dominant form of bladder, ureter, and renal pelvis cancers, comprising more than 90% of all cases. Cancer of the bladder was the most common among the three sites (>90%). Lifestyle factors to be incorporated in the analyses include smoking, occupational exposures to aromatic amines and polycyclic hydrocarbons, diet, alcohol consumption, and an index for socioeconomic status.

Background and Significance: In the 2007 incidence report, bladder cancer was observed to have the highest sex-averaged ERR/Gy of any of the solid cancers. It was also the only cancer in which ERR estimates increased with attained age (Preston D et al., *Radiat Res* 2007; 168:1–64). Japanese men have had high historical smoking rates and are more likely to have been exposed to hazardous conditions in the workplace than have Japanese women. Collectively, these factors are likely to have raised baseline rates in men compared to women, possibly in complex manners due to varying patterns of exposure. Therefore, relative radiation risk estimates, which have ignored lifestyle risks, may be differentially affected. This study will investigate the impact of these lifestyle factors on radiation risk estimates and help expand our understanding of radiation risks of cancer in organs that are susceptible to lifestyle risks.

Study Methods: A cohort analysis of incident UC occurring among LSS cohort members was performed to assess radiation effects adjusted for or modified by lifestyle factors, including smoking, diet, alcohol consumption, and education. These exposures were developed from four mailed questionnaires (1965, 1969, 1979, 1991) and clinic-based questionnaires among the AHS in the 1960s. A second study was performed using a case-cohort design among about 3,500 cohort members selected by stratified sampling who participated in a mail or clinical survey. The primary exposures of the case-cohort study were radiation, smoking, and workplace exposures to aromatic amines and polycyclic aromatic hydrocarbons. Occupational exposures were derived by abstracting occupations and industries from questionnaires and using a job exposure matrix (JEM). Survival analysis methods were used for the case-cohort analysis.

Study Progress: The cohort analysis was completed and a peer-reviewed manuscript was published in 2012. Drafts of two manuscripts for the case-cohort study have been written and circulated but additional work needs to be completed prior to submission.

Results and Conclusions: Strong independent smoking ($RR = 2.0$) and radiation effects ($ERR/Gy_w = 1.0$) were observed for UC. No effect modification or confounding was observed in the radiation effects due to smoking or other lifestyle factors. In the case-cohort analysis, similar radiation and smoking risks were observed. The point estimate for aromatic amines was greater than unity among men (1.34) but the confidence intervals were wide and not significant. No risks were observed for polycyclic aromatic hydrocarbon exposures. No modification or confounding of the radiation risks were observed. Previously reported radiation effects for bladder cancer were not seriously biased by lifestyle factors.

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

Objectives: To gain insight on the confounding or effect-modification of height, weight, and body mass index (BMI) upon the radiation exposure and colon cancer relationship. To validate the BMI from self-reported height and weight from LSS questionnaires by comparing them to measured BMI values from the AHS clinic.

Background and Significance: The role of anthropometric factors and their potentially confounding or modifying effect on radiation risk have not been adequately characterized among the atomic-bomb survivors. Previous studies have shown that radiation is associated with reduced body size in adults who were exposed to the atomic bombings as children. Though a majority of studies suggest a direct relationship between reduced caloric intake and a lower risk of cancer, studies of the Dutch famine of 1944–1945 observed a higher risk of cancer and endocrine alterations (IGF-1) associated with colon cancer risk among those exposed to

short-term malnutrition resulting from war conditions. Finally, it is well known that radiation is associated with an increased risk of colon cancer incidence (ERR/Gy: 0.54, 90% CI: 0.30–0.81) in the LSS. Therefore, it may be important to assess the role of anthropometric factors as potential confounders or effect modifiers of colon cancer radiation effects, a topic that has not been addressed before.

Study Progress: All analyses have been completed and a peer-reviewed manuscript was recently published.

Study Methods: This project employed a cohort design using all subjects enrolled in the LSS who completed at least one lifestyle questionnaire (LSS65, LSS69, LSS78, or LSS91). The primary variables of interest include height, weight, BMI, radiation dose to the colon, and incident colon cancer. Data were analyzed using Cox regression methods, with attained age as the time axis. The relative risk of colon cancer development as a function of radiation dose was obtained. We adjusted for height, weight, BMI, colon subsite (proximal/distal), and age at colon cancer onset. Additional potential confounders that were examined included smoking and alcohol use. The second phase of analyses investigated possible interactions of the effects of anthropometric factors and radiation.

Results and conclusions: Under the auspices of the RERF-University of Washington Radiation Partnership Program, this study concluded that the inclusion of anthropometric variables in models had little impact on radiation risk estimates, and there was no evidence that sensitivity to the effects of radiation on colon cancer risk depended on BMI. The earliest reported BMI, BMI reported closest to colon cancer diagnosis, and time-varying BMI all were associated with a significantly elevated risk of colon cancer (relative risk [RR] per 5 kg/m² increase in BMI = 1.12, 95% CI: 1.02–1.23; RR = 1.11, 95% CI: 1.02–1.21; and RR = 1.11, 95% CI: 1.02–1.21, respectively). Questionnaire data on BMI were validated. No confounding or effect modification of radiation effects due to BMI status was observed.

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

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

Objectives: The uncertainty in survivor dose estimates and the resulting effect on RERF studies is a longstanding concern at RERF. It is currently addressed by adjusting doses using correction factors based on regression calibration and assumptions about the probability distributions of the errors. This study intends to examine the effect of assumptions about the error distributions on important types of RERF risk estimation studies by using Bayesian models for individual data with fully specified likelihoods rather than the standard approach of Poisson regression on grouped data. For a variety of radiosensitive cancer sites, estimates obtained using Bayesian Markov Chain Monte Carlo (MCMC) methods will be compared with those obtained using the regression calibration (substitution) method, where both methods are based on individual data.

Background and Significance: A major source of uncertainty concerns extrapolation of risks at high doses and high dose rates to low doses and low dose-rates and the

impact of both systematic and random dosimetric errors on those. It is well recognized that measurement error can alter substantially the shape of this relationship and hence the derived population risk estimates.

Pierce et al. (*Radiat Res* 1990; 123:275–84) carried out a dose adjustment prior to the model fitting to allow for random dosimetric errors by substituting the expectation of the “true dose” given the estimated dose. This leads to reasonable adjusted point estimates of the model parameters for linear dose-effect relationships but is only approximate for non-linear ones, and does not fully take account of all the variability induced by the measurement errors.

The proposed Bayesian approach to the measurement error problem links three basic sub-models: the disease model, the measurement model, and the exposure model, and dosimetric uncertainty is reflected in the variability of the model parameters. In principle this allows for more of the uncertainties to be taken into account, and will produce a wider, but more realistic, uncertainty envelope. However, it is possible that in practice this may not happen to any great extent.

Study Methods: The posterior distribution of the risk parameters for the various data sets of interest will be obtained by samples from MCMC algorithms. Using individual data will allow a fully Bayesian analysis to be carried out, in particular one that embraces the full range of uncertainties, for example relating to the form of the error distribution. However, given the complexities and iterative nature of the model and the large sample size, the computational requirements are a hurdle.

Study Progress: In April 2009, Dr. Furukawa presented some initial results from simulation studies to compare a Bayesian approach for the dose error adjustment to regression calibration in Normal and logistic regression. The work initiated by Dr. Li (Imperial College London) in 2008 (nested case-control data of leukemia and thyroid cancer) made little progress in the past year due to Dr. Li's relocation.

Results and Conclusions: The computational burden was still a major issue for Cox regression on individual data, the primary goal of this project. We held discussions with Dr. Pierce about computational feasibility issues during his visit in October 2010. As he suggested, we started exploring some alternative approaches to Bayesian MCMC that may be more computationally tractable, such as multiple imputation. Some related results from a collaboration with Dr. Doi were presented at the International Biometrics Society meeting in Kobe in August, 2012.

Life Span Study Publications

RERF Reports (RR)

◆ Grant EJ, Ozasa K, Preston DL, Suyama A, Shimizu Y, Sakata R, Sugiyama H, Pham TM, Cologne JB, Yamada M, De Roos AJ, Kopecky KJ, Porter MP, Seixas N, Davis S: Effects of radiation and lifestyle factors on risks of urothelial carcinoma in the Life Span Study of atomic bomb survivors. *Radiat Res* 2012 (July); 178(1):86–98. (RR 16-11) © 2012 by Radiation Research Society

[Abstract] Among the Life Span Study (LSS) of Atomic-bomb survivors, recent estimates showed that unspecified bladder cancer had high radiation sensitivity with a

notably high female-to-male excess relative risk (ERR) per radiation dose ratio and were the only sites for which the ERR did not decrease with attained age. These findings, however, did not consider lifestyle factors, which could potentially confound or modify the risk estimates. This study estimated the radiation risks of the most prevalent subtype of urinary tract cancer, urothelial carcinoma, while accounting for smoking, consumption of fruit, vegetables, alcohol and level of education (a surrogate for socioeconomic status). Eligible study subjects included 105,402 (males = 42,890) LSS members who were cancer-free in 1958 and had estimated radiation doses. Members were censored due to loss of follow-up, incident cancer of another type, death, or the end of calendar year 2001. Surveys (by mail or clinical interview) gathered lifestyle data periodically for 1963–1991. There were 63,827 participants in one or more survey. Five hundred seventy-three incident urothelial carcinoma cases occurred, of which 364 occurred after lifestyle information was available. Analyses were performed using Poisson regression methods. The excess relative risk per weighted gray unit (the gamma component plus 10 times the neutron component, Gy_w) was 1.00 (95% CI: 0.43–1.78) but the risks were not dependent upon age at exposure or attained age. Lifestyle factors other than smoking were not associated with urothelial carcinoma risk. Neither the magnitude of the radiation ERR estimate (1.00 compared to 0.96), nor the female-to-male (F:M) ERR/ Gy_w ratio (3.2 compared to 3.4) were greatly changed after accounting for all lifestyle factors. A multiplicative model of gender-specific radiation and smoking effects was the most revealing though there was no evidence of significant departures from either the additive or multiplicative joint effect models. Among the LSS cohort members with doses greater than 0.005 Gy_w (average dose 0.21 Gy_w), the attributable fraction of urothelial carcinoma due to radiation was 7.1% in males and 19.7% in females. Among current smokers, the attributable fraction of urothelial carcinoma due to smoking was 61% in males and 52% in females. Relative risk estimates of smoking risk were approximately two for smokers compared to nonsmokers. After adjustment for lifestyle factors, gender-specific radiation risks and the F:M ERR/ Gy_w , the ratios of excess urothelial carcinoma risk were similar to the estimates without adjusting for lifestyle factors. Smoking was the primary factor responsible for excess urothelial carcinoma in this cohort. These findings led us to conclude that the radiation risk estimates of urothelial carcinoma do not appear to be strongly confounded or modified by smoking, consumption of alcohol, fruits, or vegetables, or level of education.

- ◆ Nonaka Y, Shimizu Y, Ozasa K, Misumi M, Cullings HM, Kasagi F: Application of a change point model to atomic-bomb survivor data: Radiation risk of noncancer disease mortality. *Keiryō Seibutsugaku [Jpn J Biomet]* 2012 (May); 32(2):75–96. (RR 3-11) © The Biometric Society of Japan 2012 (This abstract was reprinted by permission of the Society.)

[Abstract] The dose response of noncancer disease mortality differs by time period for atomic-bomb survivors of the Life Span Study (LSS) in the Radiation Effects Research Foundation. We applied change point models and Akaike's

Information Criteria to the data of LSS Report 13 (Preston et al. (2003)), including 86,572 subjects and 31,881 non-cancer disease deaths during 1950 to 1997. A model which incorporates a change point in the dose response but not in the background model showed results similar to those in LSS Report 13, which suggested that the baseline rate difference between proximal and distal survivors changed by time and the preferred dose response might be linear-quadratic in 1950–1967 and linear in 1968–1997. However, the present model, which incorporates change points in both dose response and background models, showed that there was little evidence to support a time-dependent change in the baseline rate difference between proximal and distal survivors. The preferred dose response was pure-quadratic in 1950–1964 and linear in 1965–1997. When the data were divided into cardiovascular disease (CVD) mortality and noncancer disease mortality other than CVD, the shape of the dose response did not differ by time period (linear for CVD and pure-quadratic for other than CVD).

- ◆ Ozasa K, Grant EJ, Cullings HM, Shore RE: Invited Commentary: Missing doses in the Life Span Study of Japanese atomic bomb survivors. *Am J Epidemiol* 2013 (February); 177(6):569–73. (RR 15-12) © The Author 2013. Published by Oxford University Press on behalf of the Johns Hopkins Bloomberg School of Public Health

[Abstract] The Life Span Study is a long-term epidemiologic cohort study of survivors of the atomic bombs dropped on Hiroshima and Nagasaki, Japan. In this issue of the *Journal*, Richardson et al. (*Am J Epidemiol*. 2013;177(6):562–568) suggest that those who died in the earliest years of follow-up were more likely to have a missing dose of radiation exposure assigned, leading to a bias in the radiation risk estimates. We show that nearly all members of the cohort had shielding information recorded before the beginning of follow-up and that much of the alleged bias that Richardson et al. describe simply reflects the geographic distribution of shielding conditions for which reliable dosimetry was impossible.

- ◆ Sakata R, Grant EJ, Ozasa K: Long-term follow-up of atomic bomb survivors. *Maturitas* 2012 (June); 72(2):99–103. (RR 2-12) © 2012 Elsevier Ireland Ltd. (This abstract was reprinted by permission of Elsevier.) (related to *Adult Health Study* and *Tumor and Tissue Registries*)

[Abstract] The Life Span Study (LSS) is a follow-up study of atomic bomb (A-bomb) survivors to investigate the radiation effects on human health and has collected data for over 60 years. The LSS cohort consists of 93,741 A-bomb survivors and another 26,580 age and sex-matched subjects who were not in either city at the time of the bombing. Radiation doses have been computed based on individual location and shielding status at the time of the bombings. Age at death and cause of death are gathered through the Japanese national family registry system and cancer incidence data have been collected through the Hiroshima and Nagasaki cancer registries. Noncancer disease incidence and health information are collected through biannual medical examinations among a subset of the LSS. Radiation significantly increases the risks of death (22% at 1 Gy), cancer incidence (47% at 1 Gy), death due to leukemia (310% at 1 Gy), as well as the

incidence of several noncancer diseases (e.g. thyroid nodules, chronic liver disease and cirrhosis, uterine myoma, and hypertension). Significant effects on maturity (e.g. growth reduction and early menopause) were also observed. Long-term follow-up studies of the A-bomb survivors have provided reliable information on health risks for the survivors and form the basis for radiation protection standards for workers and the public.

- ◆ Sakata R, McGale P, Grant EJ, Ozasa K, Peto R, Darby SC: Impact of smoking on mortality and life expectancy in Japanese smokers: A prospective cohort study. *BMJ* 2012 (October); 345:e7093. (RR 6-11)

[Abstract] Objective: To investigate the impact of smoking on overall mortality and life expectancy in a large Japanese population, including some who smoked throughout adult life. **Design:** The Life Span Study, a population-based prospective study, initiated in 1950. **Setting:** Hiroshima and Nagasaki, Japan. **Participants:** Smoking status for 27 311 men and 40 662 women was obtained during 1963–92. Mortality from one year after first ascertainment of smoking status until 1 January 2008 has been analysed. **Main outcome measures:** Mortality from all causes in current, former, and never smokers. **Results:** Smokers born in later decades tended to smoke more cigarettes per day than those born earlier, and to have started smoking at a younger age. Among those born during 1920–45 (median 1933) and who started smoking before age 20 years, men smoked on average 23 cigarettes/day, while women smoked 17 cigarettes/day, and, for those who continued smoking, overall mortality was more than doubled in both sexes (rate ratios versus never smokers: men 2.21 (95% confidence interval 1.97 to 2.48), women 2.61 (1.98 to 3.44)) and life expectancy was reduced by almost a decade (8 years for men, 10 years for women). Those who stopped smoking before age 35 avoided almost all of the excess risk among continuing smokers, while those who stopped smoking before age 45 avoided most of it. **Conclusions:** The lower smoking related hazards reported previously in Japan may have been due to earlier birth cohorts starting to smoke when older and smoking fewer cigarettes per day. In Japan, as elsewhere, those who start smoking in early adult life and continue smoking lose on average about a decade of life. Much of the risk can, however, be avoided by giving up smoking before age 35, and preferably well before age 35.

- ◆ Samartzis D, Nishi N, Cologne JB, Funamoto S, Hayashi M, Kodama K, Miles EF, Suyama A, Soda M, Kasagi F: Ionizing radiation exposure and the development of soft-tissue sarcomas in atomic-bomb survivors. *J Bone Joint Surg Am* 2013 (February); 95(3):222–9. (RR 1-11) © 2013 by the Journal of Bone and Joint Surgery, Incorporated (*This abstract was reprinted by permission of Rockwater, Inc.*) (related to *Tumor and Tissue Registries*)

[Abstract] Background: Very high levels of ionizing radiation exposure have been associated with the development of soft-tissue sarcoma. The effects of lower levels of ionizing radiation on sarcoma development are unknown. This study addressed the role of low to moderately high levels of ionizing radiation exposure in the development of soft-tissue sarcoma. **Methods:** Based on the Life Span Study cohort of Japanese atomic-bomb survivors, 80,180

individuals were prospectively assessed for the development of primary soft-tissue sarcoma. Colon dose in gray (Gy), the excess relative risk, and the excess absolute rate per Gy absorbed ionizing radiation dose were assessed. Subject demographic, age-specific, and survival parameters were evaluated. **Results:** One hundred and four soft-tissue sarcomas were identified (mean colon dose = 0.18 Gy), associated with a 39% five-year survival rate. Mean ages at the time of the bombings and sarcoma diagnosis were 26.8 and 63.6 years, respectively. A linear dose-response model with an excess relative risk of 1.01 per Gy (95% confidence interval [CI]: 0.13 to 2.46; $p = 0.019$) and an excess absolute risk per Gy of 4.3 per 100,000 persons per year (95% CI: 1.1 to 8.9; $p = 0.001$) were noted in the development of soft-tissue sarcoma. **Conclusions:** This is one of the largest and longest studies (56 years from the time of exposure to the time of follow-up) to assess ionizing radiation effects on the development of soft-tissue sarcoma. This is the first study to suggest that lower levels of ionizing radiation may be associated with the development of soft-tissue sarcoma, with exposure of 1 Gy doubling the risk of soft-tissue sarcoma development (linear dose-response). The five-year survival rate of patients with soft-tissue sarcoma in this population was much lower than that reported elsewhere. **Level of Evidence:** Prognostic Level I. See Instructions for Authors for a complete description of levels of evidence.

- ◆ Semmens EO, Kopecky KJ, Grant EJ, Mathes RW, Nishi N, Sugiyama H, Moriwaki H, Sakata R, Soda M, Kasagi F, Yamada M, Fujiwara S, Akahoshi M, Davis S, Kodama K, Li CI: Relationship between anthropometric factors, radiation exposure, and colon cancer incidence in the Life Span Study cohort of atomic bomb survivors. *Cancer Causes Control* 2013 (January); 24(1):27–37. (RR 27-11) © Springer Science+Business Media Dordrecht 2012 (*This abstract was reprinted by permission of Springer.*)

[Abstract] Purpose: We examined colon cancer risk in atomic bomb survivors to investigate whether excess body weight after the bombings alters sensitivity to radiation effects. **Methods:** Of the 56,064 Japanese atomic bomb survivors with follow-up through 2002 with self-reported anthropometric data obtained from periodic mail surveys, 1,142 were diagnosed with colon cancer. We evaluated the influence of body mass index (BMI) and height on radiation-associated colon cancer risk using Poisson regression. **Results:** We observed a similar linear dose-response relationship for the 56,064 subjects included in our analysis and the entire cohort of Japanese atomic bomb survivors [excess relative risk (ERR) per Gray (Gy) = 0.53, 95% confidence interval (CI) 0.25–0.86]. Elevation in earliest reported BMI, BMI reported closest to colon cancer diagnosis, and time-varying BMI were associated with an elevated risk of colon cancer [relative risk (RR) per 5 kg/m² increase in BMI = 1.14, 95% CI 1.03–1.26; RR = 1.16, 95% CI 1.05–1.27; and RR = 1.15, 95% CI 1.04–1.27, respectively]. Height was not significantly related to colon cancer risk. Inclusion of anthropometric variables in models had little impact on radiation risk estimates, and there was no evidence that sensitivity to the effect of radiation on colon cancer risk depended on BMI. **Conclusions:** Radiation exposure and BMI are

both risk factors for colon cancer. BMI at various times after exposure to the atomic bombings does not significantly influence the relationship between radiation dose and colon cancer risk, suggesting that BMI and radiation impact colon cancer risk independently of each other.

Other Journal Publications

- ◆ Kodama K: Epidemiological studies on cardiovascular disease in Hiroshima and Nagasaki, NI-HON-SAN Study, and training of young cardiovascular epidemiologists by conducting teaching seminar. *Nihon Junkankibyō Yobogakkaishi [Jpn J Cardiovasc Dis Prev]* 2013 (January); 48(1):42–50. (Japanese) (related to *Adult Health Study* and *Special Clinical Studies*)
- ◆ Kodama K, Ozasa K, Katayama H, Shore RE, Okubo T: Radiation effects on cancer risks in the Life Span Study cohort. *Radiat Prot Dosimetry* 2012 (October); 151(4):674–6. (related to *Special Cancer Studies* and *Tumor and Tissue Registries*)
- ◆ Nakamura N: How to tell the public about issues that are under debate. *Kankyō to Kenkō [Environ Health]* 2013 (March); 26(1):38–42. (Japanese)
- ◆ Ozasa K: Association between radiation exposure and non-cancer disease death in the atomic-bomb survivors. *Hoshasen Bogo Bunkakaishi [Jpn Soc Radiol Technol]* 2012 (October); No. 35:27–30. (Japanese) (related to *Adult Health Study*)
- ◆ Ozasa K: Effects of atomic-bomb radiation on children. *Child Health* 2012 (September); 15(9):14–7. (Japanese)
- ◆ Ozasa K: Health effects of atomic-bomb radiation. *Nippon Eiseigaku Zasshi [Jpn J Hyg]* 2013 (March); 68(Suppl):S78–9. (Abstracts of the 83rd Annual Meeting of the Japan Society for Hygiene, Kanazawa, 24–26 March 2013) (Japanese)
- ◆ Ozasa K: Late Health Effects of Radiation among the Atomic-bomb Survivors. *Nagasaki Igakkai Zasshi [Nagasaki Med J]* 2012 (September); 87(Special issue):157–60. (Proceedings of the 53rd Late A-bomb Effects Research Meeting, 2012) (Japanese)
- ◆ Ozasa K: Radiation effects at low-dose levels and effects of age at bombing among the atomic-bomb survivors. *Proceedings of the FY2011 Radiation Epidemiology Research Meeting*. Tokyo: Radiation Effects Association; 2012, pp 4–5. (Japanese)

Manuscript in Press

- ⌘ Ozasa K: Effects of radiation in atomic-bomb survivors who were young at the time of bombing. *Nihon Shoni Ketsueki/Gan Gakkai Zasshi [Jpn J Pediatr Hematol Oncol]*. (Japanese)

Life Span Study Oral Presentations

- ❖ Kodama K, Ozasa K. Epidemiological project on A-bomb survivors. 52nd Annual Meeting of the Japanese Respiratory Society, 20–22 April 2012, Kobe (related to *Tumor and Tissue Registries*)
- ❖ Shore RE, Ozasa K, Hsu WL, Sugiyama H, Furukawa K. Overview of low dose/dose-rate cancer epidemiology. 13th International Congress of International Radiation Protection Association (IRPA), 13–18 May 2012, Glasgow, Scotland
- ❖ Ozasa K. Long-term investigations of the late health

effects of atomic-bomb radiation exposure. 66th Annual Meeting of the Japanese Stomatological Society, 17–18 May 2012, Hiroshima

- ❖ Ozasa K. Late health effects of radiation among the atomic-bomb survivors. 53rd Late A-bomb Effects Research Meeting, 3 June 2012, Nagasaki
- ❖ Ozasa K. Epidemiological studies of atomic-bomb survivors and issues on the risk assessment at low-dose levels among them. 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima
- ❖ Shore RE. Highlights of research results on radiation risks. 20th World Congress of International Physicians for the Prevention of Nuclear War (IPPNW), 25 August 2012, Hiroshima (related to *Adult Health Study*)
- ❖ Furukawa K. Multiple imputation for incomplete time-dependent covariates in survival analysis. 26th International Biometric Conference, 26–31 August 2012, Kobe
- ❖ Ozasa K. Introduction of Radiation Epidemiology. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai
- ❖ Ozasa K. Estimation of radiation risk of lung cancer. 4th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 12–14 September 2012, Helsinki, Finland
- ❖ Ozasa K, Shimizu Y, Grant EJ, Sakata R, Sugiyama H, Soda M, Kodama K. The latest update on atomic-bomb survivor studies. 4th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 12–14 September 2012, Helsinki, Finland
- ❖ Ozasa K. Long-term radiation risk of cancer in the atomic-bomb survivors. 71st Annual Meeting of the Japanese Cancer Association, 19–21 September 2012, Sapporo
- ❖ Nakamura N. How to understand cancer risks at low doses. Symposium of Atomic Energy Society of Japan, 21 September 2012, Higashi-hiroshima
- ❖ Double EB, Neriishi K, Ozasa K, Hart JL. Transporting and communicating RERF risk estimates to those exposed from Fukushima. 58th Annual Meeting of the Radiation Research Society, 30 September–3 October 2012, San Juan, Puerto Rico
- ❖ Sakata R, Sugiyama H, Soda M, Grant EJ, Shimizu Y, Ozasa K. Joint effects of smoking status and radiation on stomach cancer among atomic-bomb survivors. 58th Annual Meeting of the Radiation Research Society, 30 September–3 October 2012, San Juan, Puerto Rico
- ❖ Ozasa K. Association between radiation exposure and non-cancer disease death in the atomic bomb. 40th Scientific Meeting in Autumn of the Japanese Society of Radiological Technology, 5 October 2012, Tokyo (related to *Adult Health Study*)
- ❖ Sakata R, McGale P, Grant EJ, Darby SC, Ozasa K. The Life Span Study of atomic-bomb survivors. Richard Doll Centenary Meeting, 29–31 October 2012, Oxford, UK
- ❖ Ozasa K, Shimizu Y, Takahashi I, Yamada M, Kodama K, Kasagi F, Suzuki G. Non-cancer effects (cardiovascular diseases) in Life Span Study. 3rd Science and Values Workshop and 6th Asian Regional Conference on the Evolution of the System of Radiological Protection, 6–8 November 2012, Tokyo (related to *Adult Health Study*)
- ❖ Ozasa K. Late health effects of atomic-bomb radiation. 25th International Symposium “Radiation and Cancer,” 6–8

December 2012, Tokyo

❖ Sakata R, Sugiyama H, Soda M, Grant EJ, Shimizu Y, Ozasa K. Difference in radiation effects on stomach cancer by smoking status among atomic-bomb survivors. 23rd Annual Scientific Meeting of the Japan Epidemiological Association, 24–26 January 2013, Suita

❖ Sugiyama H, Misumi M, Sakata R, Grant EJ, Shimizu Y, Soda M, Ozasa K. Mortality and radiation risk among *in utero* A-bomb survivors (1950–2008). 23rd Annual Scientific Meeting of the Japan Epidemiological Association, 24–26 January 2013, Suita

❖ Ozasa K. Late health effects of the atomic-bomb radiation. 3rd RIRBM (Hiroshima University Research Institute for Radiation Biology and Medicine) International Symposium “Biological Effects of Low Dose Radiation,” 12–13 February 2013, Hiroshima

❖ Kodama K, Ozasa K, Ohishi W, Katayama H, Okubo T. Support for Fukushima provided by the Radiation Effects Research Foundation. International Academic Conference on Radiation Health Risk Management in Fukushima, 25–27 February 2013, Fukushima (related to *Adult Health Study*)

❖ Cullings HM. The impact on the Japanese atomic-bomb survivors of radiation received from the bombs. 2013 NCRP (U.S. National Council on Radiation Protection & Measurements) 49th Annual Meeting “Radiation Dose and the Impacts on Exposed Populations,” 11–12 March 2013, Bethesda, Maryland, USA

❖ Shore RE. Radiation impacts on human health: Certain, fuzzy and unknown. 2013 NCRP (U.S. National Council on Radiation Protection & Measurements) 49th Annual Meeting “Radiation Dose and the Impacts on Exposed Populations,” 11–12 March 2013, Bethesda, Maryland, USA

❖ Ozasa K. Health effects of atomic-bomb radiation. 83rd Annual Meeting of the Japanese Society for Hygiene, 24–26 March 2013, Kanazawa

**Research Protocols 2-11, 7-10, 7-09, 3-07, 2-75 (Platform Protocol), A6-12, 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

Objectives: To test the hypothesis that arteriosclerosis develops due to abnormalities in mesenchymal tissues because cell damage from radiation exposure triggers the cytokine network involved in repair of such tissue damage in arteries.

Background and Significance: AHS data have suggested a relationship between radiation exposure and incidence of stroke and ischemic heart disease and aortic calcification. However, the developmental mechanism underlying arteriosclerosis due to radiation exposure is unclear. Some have hypothesized that arteriosclerosis is primarily an inflammatory disease, but it appears to have a complex pathology, of which inflammation is only one component. We hypothesize that atherosclerosis develops due to abnormalities in mesenchymal tissues because cell damage from radiation exposure triggers the cytokine network involved in repair of such tissue damage in arteries.

Study Methods: This is a cross-sectional study among 2,000 AHS subjects (including those exposed at young ages) in Hiroshima. We will measure several multi-functional cytokines involved in the “cytokine network” that is possibly related to radiation-induced atherogenesis and relate them to physiological measurements of atherosclerotic cardiovascular outcomes (based on RP 7-09). We will then examine if the “cytokine network” functions to either moderate or mediate the radiation effect upon atherosclerotic cardiovascular outcomes.

Study Progress: Data collection began in April 2011. We are measuring blood cytokine levels related to CVD such as pentraxin-3, osteopontin, osteoprotegerin, high mobility group box (HMGB)-1, VEGF, and apolipoprotein (Apo)-J for about 1,200 AHS subjects.

Results and Conclusions: None yet.

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

Tatsukawa Y (CH), Fujiwara S, Tamara BH, Misumi M (S), Ohishi W (CH), Yamada M (CH), Kasagi F

Objectives: The major objectives in this research protocol are to test whether radiation exposure is related to an increased incidence of arteriosclerotic diseases and their risk factors through modifications in body composition.

Background and Significance: Recent studies in childhood cancer survivors have suggested that radiation exposure causes modifications in body composition (such as higher body fat mass and lower lean mass). These findings reflect high exposures from radiotherapy (e.g., Hodgkin disease treatment), but extrapolation to lower doses is unknown. It is important to examine the association between radiation dose and body composition, and to determine

whether radiation exposure is related to an increased incidence of arteriosclerotic diseases and their risk factors partly through modifications of body composition.

Study Methods: Study subjects 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. With those data it is possible to evaluate the whole-body/regional (trunk, limb, etc.) fat mass and lean mass and to relate those to other AHS data on cardiovascular diseases.

Study Progress: We completed the analyses regarding the effects of radiation dose on modifications in body composition. The result shows A-bomb radiation dose was associated with modifications in body composition. The manuscript has been accepted by the *International Journal of Obesity*.

Results and Conclusions: A-bomb radiation dose was associated significantly and negatively with BMI in both sexes and appendicular lean mass (a surrogate marker for muscle mass). It was positively associated with trunk-to-limb fat ratio in women who were less than 15 years old at the time of exposure. In conclusion, significant associations of radiation dose with BMI and body composition were observed 50 years after A-bomb exposure.

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, Katsurada E (CH), Yamada M (CH), Ohishi W (CH), Hsu WL (S), Misumi M (S), Takahashi T, Kihara Y, Matsumoto M, Fujiwara S

Objectives: The goal is to study one of the potential mechanisms by which radiation may promote cardiovascular disease. We will evaluate acceleration of arterial stiffness by radiation among AHS subjects (including the expanded group of younger survivors).

Background and Significance: Past studies have reported a significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors.

Atherosclerosis conceptually has two aspects: atherosclerosis (fatty degeneration) and sclerosis (arterial stiffness). 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 atherosclerotic disease indices/risk factors.

Study Methods: 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 atherosclerotic disease indices (ankle-brachial blood pressure index [ABI], carotid intima-media wall thickness [IMT], aortic calcification, and left ventricular hypertrophy) and atherosclerosis risk factors (Framingham risk scores).

Study Progress: Data collection began in April 2010 and is underway. We have examined about 3,100 AHS subjects since the beginning of the study.

Results and Conclusions: None yet.

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), Ozasa K (EH), Kasagi F, Suyama A, Furukawa K (S), Cullings HM (S), Hayashi T (R), Nakachi K, Kodama Y (G), Katayama H (IT), Kodama K (CS), Nakamura N, Fujiwara S

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

Background and Significance: The existing AHS cohort consisted of all identified heavily exposed individuals and a small fraction of those lightly or moderately exposed in the age range 0–9 at the time of the bombing. Adding more of the latter to the AHS cohort provides 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.

Study Methods: 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 planned to solicit 30% of the potentially eligible who were exposed to <5 mGy, 80% of those exposed to 5 to 20 mGy, and all exposed to 20–1,000 mGy. We will focus on noncancer disease in addition to cancer.

Study Progress: We examined 1,961 survivors who were exposed at ages of 0–9 at the time of the bombings of Hiroshima and Nagasaki as of October 2010 and started a second examination in July 2010. The added study subjects will appreciably increase our ability to examine the shape of dose-response curves and estimate risks at low-to-moderate doses.

Results and Conclusions: Younger study subjects with moderate doses were successfully added to the cohort.

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

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

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 specimens and information concerning lifestyle or other potential risk factors for many fields of study, which include cytology, genetics, immunology, radiobiology, and medical dosimetry.

Background and Significance: 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 in a different environment from other unexposed study subjects. We added over 1,900 young exposed subjects (<10 years old at the bombings) to the study in 2008–2010 to increase our ability to document radiation effects among those exposed when young.

Study Methods: The study attempts to examine differences in rates of diseases or pre-clinical disorders by level of radiation exposure. During the 27th cycle (July 2010–June 2012), 2,509 individuals were examined, representing approximately 65% of the AHS cohort still living in the contact areas of interest.

Study Progress: Health examinations are continuing. The biological samples collected are used for clinical determinations and stored for ongoing and future studies. Evaluation of possible interactions have been assessed between radiation and various biological factors based on stored samples with respect to health outcomes; the factors include infectious agents, hormones, inflammatory markers, and a variety of other phenotypic and genetic factors. In addition to ongoing collaborative studies to evaluate possible interactions between radiation and infectious agents or hormones in the development of hepatocellular carcinoma, gastric cancer, and breast cancer, recently initiated collaborative studies include ones on: the association between subclinical hypothyroidism and cardiovascular risk factors, the epidemiological and genetic basis of progressive cardiac conduction defect, and weight fluctuation and cancer and cardiovascular disease incidence and mortality. The informed consent form has been updated to obtain more specific permission for preservation and later use of blood and urine samples collected at current and previous examinations.

Results: Reports of new findings include: the association of radiation exposure with fatal and non-fatal stroke, radiation dose and cataract surgery incidence, the association between chronic kidney disease and cardiovascular disease risk factors, and lipid infiltration in the parotid glands.

RP-A6-12 Choice reaction time of middle-aged and elderly Japanese as a predictor of cardiovascular mortality: Radiation Effects Research Foundation Adult Health Study

Shimizu M, Misumi M (S), Yamada M (CH), Ohishi W (CH), Yamamoto H, Kihara Y

Objectives: The association between choice reaction time and coronary heart disease (CHD) and stroke mortality in middle-aged and elderly individuals based on a roughly 35-year follow-up of participants of the Adult Health Study (AHS) will be elucidated by using Cox proportional hazards model.

Background and Significance: Reaction time, a biological index that exhibits aging-related changes and cognitive function, was reported to predict life prognosis and vascular disease mortality. However, large-scale studies of general populations and the cohort studies with broad age range were limited, and there are few reports from studies on Asian cohorts.

Study Methods: In the AHS, reaction time was measured by Bogitch's flash reaction test for about 5,000 Hiroshima participants aged 35–74 years old, between 1970 and 1972. Vital status of the subjects was followed up from the time of

baseline reaction time measurement until the end of 2007, with causes of death recorded in death certificates coded using the International Classification of Diseases (ICD). The association between reaction time and circulatory disease mortality will be investigated.

Study Progress: The dataset for analysis has been prepared.

Results and Conclusions: None yet.

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, Ohishi W (CH), Tatsukawa Y (CH), Nakashima E (S), Hsu WL (S), Misumi M (S), Fujiwara S, Akahoshi M (CN)

Objectives: To clarify whether radiation is associated with chronic kidney disease (CKD) and whether CKD is involved in the mechanism(s) linking radiation exposure and cardiovascular disease (CVD).

Background and Significance: The association between atomic-bomb radiation exposure and CVD has recently been drawing attention. 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 to examine whether CKD serves as a CVD risk factor and the possible involvement of A-bomb radiation exposure in the disease process.

Study Methods: In this analysis, we will identify prevalent cases of CKD diagnosed during the four-year period between January 1988 and December 1991 (Baseline period) and incident cases of CKD diagnosed between January 1992 and December 2006 (Follow up period) 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 in this RP. 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 CKD prevalence or incidence with radiation dose and CKD risk factors.
- (2) Associations of CHD and stroke prevalence with radiation dose, CKD risk factors and previous prevalent or incident CKD.

Study Progress: The data to analyze the association of prevalent cases of CKD with radiation dose and CKD risk factors have been collected but the incidence data on both CKD and CVD need to be compiled and cleaned.

Results and Conclusions: None yet. Results expected in 2014.

Adult Health Study Publications

RERF Reports (RR)

◆ Cologne JB, Hsu WL, Abbott RD, Ohishi W, Grant EJ, Fujiwara S, Cullings HM: Proportional hazards regression in epidemiologic follow-up studies: An intuitive consideration of primary time scale. *Epidemiology* 2012 (July); 23(4):565–73. (RR 12-11) © 2012 by Lippincott Williams & Wilkins (This abstract was reprinted by permission of Wolters Kluwer Health Medical Research.) (related to *Special Clinical Studies*)

and *Tumor and Tissue Registries*)

[Abstract] In epidemiologic cohort studies of chronic diseases, such as heart disease or cancer, confounding by age can bias the estimated effects of risk factors under study. With Cox proportional-hazards regression modeling in such studies, it would generally be recommended that chronological age be handled nonparametrically as the primary time scale. However, studies involving baseline measurements of biomarkers or other factors frequently use follow-up time since measurement as the primary time scale, with no explicit justification. The effects of age are adjusted for by modeling age at entry as a parametric covariate. Parametric adjustment raises the question of model adequacy, in that it assumes a known functional relationship between age and disease, whereas using age as the primary time scale does not. We illustrate this graphically and show intuitively why the parametric approach to age adjustment using follow-up time as the primary time scale provides a poor approximation to age-specific incidence. Adequate parametric adjustment for age could require extensive modeling, which is wasteful, given the simplicity of using age as the primary time scale. Furthermore, the underlying hazard with follow-up time based on arbitrary timing of study initiation may have no inherent meaning in terms of risk. Given the potential for biased risk estimates, age should be considered as the preferred time scale for proportional-hazards regression with epidemiologic follow-up data when confounding by age is a concern.

◆ Neriishi K, Nakashima E, Akahoshi M, Hida A, Grant EJ, Masunari N, Funamoto S, Minamoto A, Fujiwara S, Shore RE: Radiation dose and cataract surgery incidence in atomic bomb survivors, 1986–2005. *Radiology* 2012 (October); 265(1):167–74. (RR 14-11) © RSNA, 2012 (*This abstract was reprinted by permission of Radiological Society of North America.*) (related to *Special Clinical Studies*)

[Abstract] Purpose: To examine the incidence of clinically important cataracts in relation to lens radiation doses between 0 and approximately 3 Gy to address risks at relatively low brief doses. **Materials and methods:** Informed consent was obtained, and human subjects procedures were approved by the ethical committee at the Radiation Effects Research Foundation. Cataract surgery incidence was documented for 6,066 atomic bomb survivors during 1986–2005. Sixteen risk factors for cataract, such as smoking, hypertension, and corticosteroid use, were not confounders of the radiation effect on the basis of Cox regression analysis. Radiation dose-response analyses were performed for cataract surgery incidence by using Poisson regression analysis, adjusting for demographic variables and diabetes mellitus, and results were expressed as the excess relative risk (ERR) and the excess absolute risk (EAR) (ie, measures of how much radiation multiplies [ERR] or adds to [EAR] the risk in the unexposed group). **Results:** Of 6,066 atomic bomb survivors, 1,028 underwent a first cataract surgery during 1986–2005. The estimated threshold dose was 0.50 Gy (95% confidence interval [CI]: 0.10 Gy, 0.95 Gy) for the ERR model and 0.45 Gy (95% CI: 0.10 Gy, 1.05 Gy) for the EAR model. A linear-quadratic test for upward curvature did not show a significant quadratic effect for either the

ERR or EAR model. The linear ERR model for a 70-year-old individual, exposed at age 20 years, showed a 0.32 (95% CI: 0.09, 0.53)* excess risk at 1 Gy. The ERR was highest for those who were young at exposure.

Conclusion: These data indicate a radiation effect for vision-impairing cataracts at doses less than 1 Gy. The evidence suggests that dose standards for protection of the eye from brief radiation exposures should be 0.5 Gy or less. (* The original report contained erroneous confidence intervals. This abstract shows the corrected figures. [RERF])

◆ Sakata R, Grant EJ, Ozasa K: Long-term follow-up of atomic bomb survivors. *Maturitas* 2012 (June); 72(2):99–103.

(RR 2-12) (related to *Life Span Study* and *Tumor and Tissue Registries*) (refer to abstract in *Life Span Study Publications*)

◆ Sera N, Hida A, Imaizumi M, Nakashima E, Akahoshi M: The association between chronic kidney disease and cardiovascular disease risk factors in atomic bomb survivors. *Radiat Res* 2013 (January); 179(1):46–52. (RR 20-11) © 2013 by Radiation Research Society

[Abstract] Atomic bomb (A-bomb) radiation is associated with cardiovascular disease (CVD) and metabolic CVD risk factors. Chronic kidney disease (CKD) is also known to be a risk factor for CVD and little is known whether CKD is associated with A-bomb radiation. To examine whether CKD is associated with CVD risk factors or with A-bomb radiation in A-bomb survivors, we classified renal dysfunction in 1,040 A-bomb survivors who were examined in 2004–2007 as normal [$n = 121$; estimated glomerular filtration rate (eGFR) ≥ 90 ml/min/1.73 m²]; mild ($n = 686$; eGFR 60–89 ml/min/1.73 m²); moderate ($n = 217$; eGFR 30–59 ml/min/1.73 m²); or severe ($n = 16$; eGFR < 30 ml/min/1.73 m²). Also, we diagnosed subjects in the moderate and severe renal dysfunction groups as having CKD ($n = 233$; eGFR < 59 ml/min/1.73 m²). After adjusting for age, gender, and smoking and drinking habits, we looked for an association between renal dysfunction and hypertension, diabetes mellitus (DM), hyperlipidemia, and metabolic syndrome (MetS), and between renal dysfunction and A-bomb radiation. Hypertension [odds ratio (OR), 1.57; 95% confidence interval (CI), 1.12–2.20, $P = 0.009$]; DM (OR, 1.79; 95% CI, 1.23–2.61, $P = 0.002$); hyperlipidemia (OR, 1.55; 95% CI, 1.12–2.14, $P = 0.008$); and MetS (OR, 1.86; 95% CI, 1.32–2.63, $P < 0.001$) were associated with CKD (moderate/severe renal dysfunction), and hyperlipidemia and MetS were also associated with mild renal dysfunction. CKD (OR/Gy, 1.29; 95% CI, 1.01–1.63, $P = 0.038$) and severe renal dysfunction (OR/Gy, 3.19; 95% CI, 1.63–6.25, $P < 0.001$) were significantly associated with radiation dose. CKD associated with radiation may have played a role in the development of CVD among A-bomb survivors.

◆ Takahashi I, Furukawa K, Ohishi W, Takahashi T, Matsumoto M, Fujiwara S: Comparison between oscillometric- and Doppler-ABI in elderly individuals. *Vasc Health Risk Manag*; 2013 (March); 9:89–94. (RR 3-12) © 2013 Takahashi et al., publisher and licensee Dove Medical Press Ltd.

[Abstract] Peripheral arterial disease (PAD) generally remains under-recognized, mainly due to the specialized technical skills required to detect the low values of the ankle-brachial index (ABI). As a simpler and faster

alternative to the standard method using continuous-wave Doppler ultrasound, we evaluated automated oscillometric ABI measurement by VP-2000 with an elderly cohort of 113 subjects (age range, 61 to 88 years). The standard deviation in ABIs measured by the Doppler method was statistically greater than that measured by the oscillometric method for each of the two legs ($P < 0.001$). Correlations in ABIs between the two methods were 0.46 for the left leg and 0.61 for the right leg; this result appears to have been caused by interobserver variation in the Doppler ABI measurements. While the trend showing greater differences between average oscillometric- and Doppler-ABIs was significant at the lower ABI ranges, there was little indication of differences in measurements having an average ABI > 1.1 . The difference between the methods was suggestively larger in subjects who were smokers than in non-smokers ($P = 0.09$), but the difference was not affected by other potential atherosclerotic risk factors, including age at examination ($P > 0.50$). A larger difference at lower ABIs led to better PAD detection by the Doppler method compared to the oscillometric method (sensitivity = 50%, specificity = 100%), although the overall agreement was not small (Cohen's Kappa = 0.65). Our findings indicate that oscillometric devices can provide more accurate estimation of the prevalence of PAD in elderly individuals than the conventional Doppler method.

◆ Yamada M, Shimizu M, Kasagi F, Sasaki H: Reaction time as a predictor of mortality: The Radiation Effects Research Foundation Adult Health Study. *Psychosom Med*; 2013 (January); 75(2-3):154-60. (RR 25-11) © 2013 by the American Psychosomatic Society (This abstract was reprinted by permission of Wolters Kluwer Health Medical Research.) (related to *Special Clinical Studies*)

[Abstract] Objective: We investigated the association between reaction time (RT) and mortality in middle-aged and older atomic bomb survivors and their unexposed controls over a period of 30 years. **Methods:** During 1970-72, 4912 participants of the Adult Health Study cohort in Hiroshima, Japan, underwent biologic tests including RT. Mortality was followed to the end of 2003. **Results:** In a multivariate-adjusted model, the hazard ratio (HR) for 1-standard deviation increments of RT was 1.08 (95% confidence interval [CI] = 1.03-1.13) for men, 1.22 (95% CI = 1.16-1.28) for women, and 1.13 (95% CI = 1.09-1.16) for all. When the analysis was performed by sex, age, and follow-up period, a consistent increase of mortality with increments of RT was observed. The HR for mortality for the highest RT quintile was higher than that of the lowest quintile in all sex-age groups. A significant positive association between mortality risk and RT was observed even after 20 years of follow-up ($p = .03$ in men, $p < .001$ in women). RT and radiation dose were risk factors for mortality independent of conventional risk factors such as smoking, high blood pressure, and diabetes mellitus. Interaction between RT and radiation dose had no significant effect on mortality in men. Although increased radiation dose reduced the HR for mortality per RT increment in women, RT and radiation dose were still significant predictors of mortality. **Conclusions:** RT is a consistently strong predictor of mortality. Although mortality risk increased with radiation

dose, radiation did not accelerate the relationship between RT and mortality. (Note: All deaths not caused by external causes were included in this study. [RERF])

Other Journal Publications

◆ Kodama K: Epidemiological studies on cardiovascular disease in Hiroshima and Nagasaki, NI-HON-SAN Study, and training of young cardiovascular epidemiologists by conducting teaching seminar. *Nihon Junkankibyō Yobogakkaishi [Jpn J Cardiovasc Dis Prev]* 2013 (January); 48(1):42-50. (Japanese) (related to *Life Span Study* and *Special Clinical Studies*)

◆ Kodama K: Radiation health effects: radiation and cancer risk in atomic bomb survivors. *Medical Science Digest* 2012 (November); 38(13):20-3. (Japanese) (related to *Tumor and Tissue Registries*)

◆ Ozasa K: Association between radiation exposure and non-cancer disease death in the atomic-bomb survivors. *Hoshasen Bogo Bunkakaishi [Jpn Soc Radiol Technol]* 2012 (October); No. 35:27-30. (Japanese) (related to *Life Span Study*)

Manuscript in Press

⌘ Tatsukawa Y, Misumi M, Yamada M, Masunari N, Oyama H, Nakanishi S, Fukunaga M, Fujiwara S: Alteration of body mass index and body composition in atomic bomb survivors. *Int J Obesity*.

Adult Health Study Oral Presentations

❖ Tatsukawa Y, Misumi M, Yamada M, Nakanishi S, Fujiwara S. Body composition and radiation exposure in the Adult Health Study. 15th International Congress of Endocrinology, 5-9 May 2012, Florence, Italy

❖ Tatsukawa Y, Yamada M, Nakanishi S, Fujiwara S. Review of association of body fat distribution with prevalence of diabetes and metabolic syndrome. 55th Annual Scientific Meeting of the Japan Diabetes Society, 17-19 May 2012, Yokohama (related to *Special Clinical Studies*)

❖ Takahashi I, Hida A, Akahoshi M, Fujiwara S. Association between AI-central blood pressure and height loss starting in middle age. 12th Clinical Conference of Blood Pressure and Pulse Wave, 9 June 2012, Tokyo

❖ Takahashi I, Fujiwara S. Association between height loss and augmentation index. 3rd Conference of Osteobioscience, 14 July 2012, Okayama (related to *Special Clinical Studies*)

❖ Shore RE. Highlights of research results on radiation risks. 20th World Congress of International Physicians for the Prevention of Nuclear War (IPPNW), 25 August 2012, Hiroshima (related to *Life Span Study*)

❖ Tatsukawa Y, Misumi M, Yamada M. Relationship between atomic-bomb radiation exposure and obesity: Hiroshima Adult Health Study. 55th Annual Meeting of the Japan Radiation Research Society, 6-8 September 2012, Sendai

❖ Ozasa K. Association between radiation exposure and non-cancer disease death in the atomic bomb. 40th Scientific Meeting in Autumn of the Japanese Society of Radiological Technology, 5 October 2012, Tokyo (related to *Life Span Study*)

❖ Ozasa K, Shimizu Y, Takahashi I, Yamada M, Kodama K, Kasagi F, Suzuki G. Non-cancer effects (cardiovascular

diseases) in Life Span Study. 3rd Science and Values Workshop and 6th Asian Regional Conference on the Evolution of the System of Radiological Protection, 6–8 November 2012, Tokyo (related to *Life Span Study*)

❖ Takahashi I. Assessment of height loss on cardiovascular system. 24th Chugoku District Meeting of the Japan Geriatric Society, 24 November 2012, Hiroshima (related to *Special Clinical Studies*)

❖ Tatsukawa Y, Yamada M, Nakanishi S. Regional body fat distribution and prevalence of diabetes. 9th International Diabetes Federation Western Pacific Region Congress and 4th Scientific Meeting of Asia Association for the Study of Diabetes, 24–29 November 2012, Kyoto (related to *Special Clinical Studies*)

❖ Akahoshi M. Effects of radiation exposure. Joint Symposium on the 20th Anniversary of the NASHIM and the 50th Anniversary of the Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 10 February 2013, Nagasaki

❖ Kodama K, Ozasa K, Ohishi W, Katayama H, Okubo T. Support for Fukushima provided by the Radiation Effects Research Foundation. International Academic Conference on Radiation Health Risk Management in Fukushima, 25–27 February 2013, Fukushima (related to *Life Span Study*)

Research Protocols 4-10 (Platform Protocol), A3-12 F₁ Clinical Study

RP 4-10 Longitudinal clinical study of the F₁ offspring of A-bomb survivors

Ohishi W (CH), Fujiwara S, Tatsukawa Y (CH), Akahoshi M (CN), Suyama A, Furukawa K (S), Hsu WL (S), Takahashi N, Satoh Y (G), Kusunoki Y (R), Yamada M (CH), Takahashi I (CH), Ueda K (CH), Katsurada E (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

Objectives: The objective of this study is to elucidate the effects of parental exposure to A-bomb radiation on the development of polygenic, multifactorial diseases and subclinical conditions among the F₁ offspring.

Background and Significance: The previous cross-sectional F₁ clinical study from 2002 to 2006 provided no evidence for an increased prevalence of adult-onset multifactorial diseases due to parental radiation exposure, but the study subjects were still quite young. The rationale for this study is that definitive human data can be obtained only if a high-quality clinical study is continued until the subjects become elderly, when many multifactorial diseases develop. Self-selection bias also tends to be minimized when prospective longitudinal data are obtained, because such data allow estimates of disease incidence.

Study Methods: This prospective study will conduct quadrennial health examinations for up to 12,500 subjects who responded favorably by mail or telephone to participate in health examinations between May 2000 and November 2008. Multifactorial diseases detected via these examinations will be examined in relation to radiation exposure of their parents, taking into consideration confounding factors.

Study Progress: We have started the longitudinal F₁ clinical study in November 2010. We sent brochures that provide an overview of the health examinations to 6,783 potential study subjects for the past year and have contacted them by telephone to request their participation in the health examination. Of those, 4,886 subjects have undergone health examinations, and 174 subjects are due to participate in health examinations. FY2012 participation rate (74.2%) showed an increase of 5.0% compared with FY2011 (69.2%). Among those who were examined in 2002–06, 80.3% are participating again. A paper regarding genetic effects on individual multifactorial diseases based on the previous cross-sectional F₁ clinical study was published electronically in *Journal of Radiological Protection*, and its hard copy version is currently in press.

Results and Conclusions: The previous cross-sectional F₁ clinical study provided no evidence of a risk from parental radiation exposure for hypercholesterolemia, hypertension, diabetes, angina, myocardial infarction, or stroke when the various endpoints were examined individually.

RP-A3-12 Continued preservation of frozen fresh thyroid samples obtained from children of A-bomb survivors

Imaizumi M (CN), Ohishi W (CH), Sera N (CN), Hida A (CN), Yamada M (CH), Hamatani K (R), Akahoshi M (CN)

Objectives: The purpose of the present study is continued

preservation of the existing frozen fresh thyroid tissues from thyroid cancer cases detected among the subjects of the Health Effects Study of the Children of A-bomb Survivors in preparation for future molecular studies.

Background and Significance: Thyroid cancer is one of the cancers affected by radiation among A-bomb survivors. Even though evidence linking parental radiation exposure with solid cancer incidence among the offspring of A-bomb survivors has proved elusive, these offspring are just now entering their cancer-prone years. It is necessary to consider the possibility that future epidemiological research may suggest such effects. Furthermore, mechanisms behind thyroid cancer development are not yet fully understood. Under the present circumstances, preservation of as many frozen thyroid cancer samples as possible is indispensable for future molecular research of thyroid carcinogenesis. At RERF, thyroid ultrasound screening performed in the health effects study of the children of A-bomb survivors during the period 2002–2006 detected dozens of cases of thyroid cancer, which were then referred for surgical removal. From among those cases, frozen fresh thyroid samples from 36 cases are currently stored at RERF. Continued preservation of the relevant frozen fresh thyroid samples will be valuable for future molecular research.

Study Methods: We continue to store the frozen fresh thyroid samples using liquid nitrogen. Sample data (research identification number, date of surgery, site [tumor or tumor-free], quantity of sample) have been stored in a database.

Study Progress: We are continuing to store the frozen fresh thyroid samples.

Results and Conclusions: None. This RP is only for continued preservation of frozen fresh samples from thyroid cancer cases detected among the F₁ population in preparation for future molecular research. A research protocol using the samples will be separately prepared in the future.

F₁ Clinical Study Publication

Manuscript in Press

⌘ Tatsukawa Y, Cologne JB, Hsu WL, Yamada M, Ohishi W, Hida A, Furukawa K, Takahashi N, Nakamura N, Suyama A, Ozasa K, Akahoshi M, Fujiwara S: Radiation risk of individual multifactorial diseases in offspring of the atomic-bomb survivors: A clinical health study. *J Radiol Prot.*

Research Protocols 5-09, 4-09, 3-09, 4-04 and 5-04, 1-03, 4-02, 2-97, 2-90, 7-87 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), 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 N-P, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

Objectives: To delineate the long-term consequences of prior A-bomb irradiation and advancing age on homeostatic control of HSCs and DCs, we will analyze numerical and functional changes within the circulating HSC and DC pools among A-bomb survivors.

Background and Significance: There is accumulating evidence that suggests accelerated immunosenescence in A-bomb survivors. However, the mechanisms of radiation-associated immunosenescence are poorly understood. We hypothesize that radiation exposure induces premature aging of HSCs, resulting in reduced numbers and impaired self-renewal ability, that in turn accelerates 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. Animal models will also be explored for a better understanding of the reconstitution of hematopoietic and immune systems following radiation-induced damage.

Study Methods: Numerical and functional changes are analyzed in relation to radiation dose 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 obtained in the A-bomb survivor studies, we have developed a series of *in vitro* assay systems and mouse experimental models to determine the functional and differentiation status of HSC and DC populations following exposure to ionizing irradiation.

Study Progress: With successfully established microimmunoassay systems, we have completed measurements of functions of circulating HSCs and DCs among about a half of study subjects (target number of subjects: 258 for HSCs; and 259 for DCs). We also submitted one manuscript on age-dependent decreases in the T-cell progenitor frequency analyzed for in-house volunteers. One paper on whole-body irradiation effects on the function of human HSCs reconstituted in NOD/Shi-*scid*, IL2R γ^{null} (NOG) immunodeficient mice was published.

Results and Conclusions: The differentiation potential of aging human HSCs may be biased toward NK relative to T cells. The humanized mouse model is useful for the evaluation of the radiation effects on human hematopoiesis.

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), Ozasa K (EH), Geyer SM, Hirabayashi Y, Iwama A, Koyasu S, Yasutomo K, Inoue T,

Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Douple EB (ACR), Nakachi K

Objectives: It is important to examine whether the radiation-impaired immune system modifies health-related status, in this case the vaccine response, in aging A-bomb survivors. 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.

Background and Significance: RERF's epidemiology and clinical studies have long indicated increased risks of age-related and immunity/inflammation-related diseases among A-bomb survivors. Further, the noted radiation effects on the immune system are similar to those associated with aging.

Study Methods: On the basis of a pilot study in FY2010 with 50 AHS subjects, a full-scale study is being conducted in FY2011 and FY2012 with 300 additional AHS subjects, who will be selected by stratified random sampling of dose group, age group, and gender. The primary endpoint is anti-influenza virus antibody titer levels three weeks after vaccination, compared with those before vaccination. Secondary endpoints include plasma levels of cytokines and inflammation-related proteins, lymphocyte subsets, and intracellular activation markers.

Study Progress: In the second year of the full-scale study, 137 eligible AHS subjects agreed to participate in this study. A total of 126 attending physicians were identified for the selected 137 subjects and showed willingness to cooperate with this study.

Results and Conclusions: The first year of the full-scale study was successfully conducted with blood samples obtained before and three weeks after vaccination from 157 AHS subjects and 23 younger in-house volunteers (controls). The effects of age on antibody titer levels in pre- and post-vaccination were examined. The hemagglutination inhibition (HI) titers against A/H1N1 and A/H3N2 antigens at pre-vaccination did not change with age, while those against B antigens at pre-vaccination significantly increased with increasing age. The HI titers against A/H1N1, A/H3N2, and B at post-vaccination tended to decrease with increasing age, although there was no statistical significance. The ratios for HI titers of these three vaccine antibodies between post- and pre-vaccination also tended to decrease with increasing age although there was no statistical significance.

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

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

Objectives: The objective of this study is to develop an integrated scoring system for evaluating the immunological and inflammatory status of individuals as a function of age and radiation dose, and predicting the effects of radiation on the immune system and somatic mutation in exposed

subjects.

Background and Significance: This immunology study, unique to RERF, consists of repeated observations of various immunological parameters in A-bomb survivors with long-term follow-up, which may demonstrate significant radiation-related alterations in the immune system among the survivors, even 65 years after the atomic bombings.

Study Methods: A cross-sectional analysis is underway that will include about 3,600 Hiroshima AHS subjects. Measurement of immunological and inflammation-related markers will be conducted. A longitudinal analysis will then be conducted on a subset of 300 selected AHS subjects. Biomarkers will be measured using antibody chip arrays on two sets of plasma samples collected from the 300 AHS subjects 10 years apart. 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.

Study Progress: We have measured plasma levels of 27 biomarkers including cytokines, chemokines, and growth factors (IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, eotaxin, TNF- α , IFN- γ , IP-10, MCP-1, MIP-1 α , MIP-1 β , RANTES, G-CSF, GM-CSF, PDGF-BB, basic-FGF, and VEGF) in 2,052 AHS subjects by the Bio-Plex Pro cytokine assay.

Results and Conclusions: The preliminary results suggested that plasma levels of IL-1 β , IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-17, eotaxin, TNF- α , IFN- γ , IP-10, MIP-1 α , G-CSF, GM-CSF, basic-FGF, and VEGF significantly increased with age after adjusting for gender. On the other hand, those of IL-9, IL-15, MIP-1 β , RANTES, and PDGF-BB decreased with age.

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), Ito R (R), Cologne JB (S), Imai K (R), Ohishi W (CH), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Nakachi K

Objectives: This study aims to assess whether genetic backgrounds of individuals affect their susceptibility to cancer, particularly as this background may be a modifier of the radiation effects.

Background and Significance: Epidemiological studies have demonstrated long-lasting impacts of A-bomb radiation on 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. Therefore, we are investigating relationships among risks of radiation-associated cancers, individuals' genetic backgrounds, and radiation exposure.

Study Methods: Using DNA extracted from stored lymphocytes and paper discs from 4,690 individuals including 1,444 cancer cases, a subcohort of the AHS cohort, we have been conducting a series of case-cohort studies of various radiation-associated cancers in terms of polymorphisms of immune/inflammation-related genes and other cancer-associated genes such as DNA repair genes and drug-

metabolizing enzyme genes. In this study, risks of cancers are evaluated as functions of both radiation dose and genotypes.

Study Progress: We investigated *CD14* and *IL18* genotypes in 194 colon and rectal cancer cases (CC and RC) and a subcohort of 2,132 individuals, calculating relative risk (RR) of the cancers for combinations of genotypes and radiation dose. CC showed an increased risk for radiation (RR/Gy = 1.16, 95% CI: 1.01–1.33), while RC did not. When the study subjects were divided into four genotype groups, i.e., two *CD14* genotypes (*CD14-A/A* and *CD14-others*) combined with two *IL-18* genotypes (*IL18-C/C* and *IL18-others*), survivors with *CD14-A/A* and *IL18-C/C* revealed further increased CC risk (RR = 3.88, 95% CI: 1.82–8.31) for the highest dose category (≥ 0.7 Gy), compared to non-exposed survivors with *CD14-others* and *IL18-others*.

Results and Conclusions: *CD14*- and *IL18*-related inflammatory responses may be involved in development of CC but not RC among survivors, and host responses to radiation may vary according to *CD14* and *IL18* genotypes.

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

Hayashi T (R), Nakashima E (S), Tatsukawa Y (CH), Imai K (R), Ohishi W (CH), Yoshida K (R), Kusunoki Y (R), Akahoshi M (CN), Nakachi K

Objectives: This study aims to assess the effect of radiation and various genetic factors on the development of diabetes mellitus (DM) in the AHS cohort, and determine whether differences in frequencies of any particular genotypes between Hiroshima and Nagasaki survivors may account for why a significant association between risk of DM and radiation dose is observed in A-bomb survivors in Hiroshima but not Nagasaki.

Background and Significance: Although early studies did not show associations between radiation exposure and DM, data on AHS subjects in 1992–1994 indicated a significant positive radiation-DM association in Hiroshima but not in Nagasaki, after adjusting for gender, 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 the immune system and that the radiation-DM association is especially relevant to a subgroup of A-bomb survivors who have a specific *HLA* class II haplotype.

Study Methods: We investigated the relationships between *HLA*-related genetic factors, risk of DM, and radiation dose in the AHS subjects in terms of a case-control study. A total of 3,419 AHS subjects, comprising DM cases (Hiroshima 597 and Nagasaki 316 subjects) and controls (Hiroshima 1,194 and Nagasaki 1,264 subjects), were selected by the Department of Statistics and genotyped on the basis of *HLA* and *HLA*-related gene polymorphisms.

Study Progress: We have determined *DRB1/DQB1/DQA1* and *A/B/C* genotypes for 824 and 725 DM cases and 2,084 and 1,900 control subjects, respectively, as well as *TP53 Arg72Pro* genotypes for 863 DM cases and 1,631 control subjects.

Results and Conclusions: None yet. Data analyses are

underway to determine associations between *HLA*-related genetic factors, radiation dose, and DM risk. Results will be obtained in 2015.

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

Kusunoki Y (R), Yoshida K (R), Kajimura J (R), Hayashi T (R), Imai K (R), Ohishi W (CH), Kodama Y (G), Nakachi K

Objectives: In this study we test the hypothesis that radiation exposure may perturb T-cell homeostasis, consequently elevating risks of various diseases.

Background and Significance: Aging of the T-cell immune system is believed to be a primary cause of increased morbidity from selected infectious and inflammatory diseases. Accumulating evidence includes: (i) T-cell repertoire deviation and clonal expansion in memory T cells, (ii) decreased T-cell function and enhanced inflammation, (iii) decreased naïve T-cell pools, and (iv) increased T-cell populations with compromised/senescent phenotype. So far, we found dose-dependent reductions in sizes of both naïve CD4 and CD8 T-cell populations, as well as increased percentages of functionally-weak memory CD4 T-cell subsets and regulatory T cells with increased radiation dose among A-bomb survivors. We also found inverse associations between plasma levels of inflammatory cytokines and the relative frequency of naïve CD4 T cells. Those results are consistent with the above hypothesis. We will also examine relationships between aging-related T-cell profiles, which had been measured at baseline, and subsequent disease development.

Study Methods: Among the AHS subjects, we will analyze 1) the percentages of peripheral blood lymphocyte subsets by flow cytometry, 2) the numbers of T-cell receptor-rearrangement excision circles (TREC) in CD4 and CD8 T-cell fractions by real-time PCR, 3) the average length of telomere repeats in both naïve and memory T-cell populations by flow FISH, and 4) associations between the T-cell parameters and disease development and/or progression. Using murine inflammation models, immune/inflammatory and genome damage biomarkers will be assessed to understand the mechanisms of radiation-induced immunological alterations and disease development.

Study Progress: TREC-bearing T-cell numbers, an indicator of individual capacity to produce new T cells, were measured among about 1,000 A-bomb survivors until FY2009. The average telomere lengths of T lymphocytes in individual survivors had also been measured. Preliminary data analyses of radiation effects on TREC numbers and telomere lengths are now underway.

Results and Conclusions: Accumulating evidence for radiation-associated changes in T-cell immunity suggests that ionizing irradiation might contribute to an enhanced T-cell immunosenescence resulting in elevated inflammatory responses and development/progression of aging-associated inflammatory diseases.

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

Hayashi T (R), Yoshida K (R), Kusunoki Y (R), Ohishi W (CH), Akahoshi M (CN), Nakachi K

Objectives: The purpose of this study is to supplement RP 2-90 by providing a means of storing DNA from stock sources that otherwise would be wasted so that the DNA would be available for multiple small-scale molecular analyses (i.e., biospecimens from RP 2-90).

Background and Significance: In studying the late effects of radiation exposure in A-bomb survivors, analyses of gene alterations due to radiation are essential in investigations of genomic instability, genetic susceptibility, and molecular oncology. With new technologies, investigations can now be conducted with extremely small amounts of DNA.

Study Methods: Blood samples, destined for DNA extraction and subsequent molecular analyses, are lyophilized and stored for use in current and future studies.

Study Progress: We have thus far cryopreserved 21,679 and 13,596 blood sample vials from AHS participants in Hiroshima and Nagasaki, respectively, including the expanded AHS participants who were exposed to radiation at the age of nine or younger. The PCR amplification of DNA extracted from laboratory control blood stored at -80°C for 15 years on paper showed no recognizable degradation of DNA after the long-term storage.

Results and Conclusions: This project provides a valuable resource for future molecular and genetic studies of radiation effects.

RP 2-90 Cryopreservation of blood cells from Hiroshima and Nagasaki Adult Health Study participants

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Ohishi W (CH), Nakachi K

Objectives: The purpose of this study is to cryopreserve viable blood mononuclear cells from all AHS participants to ensure that appropriate materials will be available for ongoing studies of the late effects of human exposure to A-bomb radiation as well as future analyses using advanced techniques.

Background and Significance: Various aspects of the medical effects of human exposure to A-bomb radiation have been studied at ABCC-RERF. It is reasonable to expect that technological improvements in analytical methodologies will continue and that studies of effects that were previously not practical will become feasible in the future.

Study Methods: Peripheral blood mononuclear cells (PBMC) are separated from 4 ml of heparinized blood using the Ficol/Hypaque density gradient centrifugation technique. PBMC are preserved in a tank of liquid nitrogen.

Study Progress: We have cryopreserved blood cells from Hiroshima and Nagasaki AHS participants. We have also collected lymphocyte samples from the expanded group of A-bomb survivors since October 2008 (those who were exposed to radiation at age of nine years or younger).

Results and Conclusions: In FY2012, we have cryopreserved blood cells from 1,259 AHS participants from Hiroshima and 800 from Nagasaki, including the expanded group members who were exposed to radiation at the age of nine years or younger. We confirmed that the viability of cryopreserved cells was more than 80% and that thawed lymphocytes expressed normal surface antigens and immunological functions.

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)

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Akahoshi M (CN), Ohishi W (CH), Nakachi K

Objectives: The initial purpose of this project was to cryopreserve Epstein-Barr virus (EBV)-transformed B-cell lines from high-dose and control survivors for new cell biology studies, e.g., those dealing with radiosensitivity. The resulting B-cell lines are also of considerable use in studying radiation effects related to immune functions and the role of genetic background in disease development.

Background and Significance: According to the recommendation of the Multinational Peer Review Panel for the radiobiology program (1998), we have been cryopreserving EBV-transformed B-cell lines from high-dose (≥ 1 Gy) and control A-bomb survivors for molecular epidemiological, genetic polymorphism, immunological, and other genomic and proteomic studies. About 500 samples that overlap with the F₁ study have been immortalized and are being stored in the Department of Genetics.

Study Methods: The AHS subjects for this study are the high dose (≥ 1 Gy) and control (< 0.005 Gy) groups. The total number of participants in those groups is about 3,500 in Hiroshima and Nagasaki. Peripheral blood lymphocytes from A-bomb survivors are being transformed by EBV and cryopreserved in liquid nitrogen.

Study Progress: We have completed EBV transformation of mononuclear cells from 2,743 AHS subjects, including nearly all who are currently participating in the AHS.

Results and Conclusions: The immortalization of lymphocytes from Hiroshima subjects is nearing completion (1,887 subjects) and lymphocytes from 856 Nagasaki AHS participants have also been successfully transformed. About 1,300 samples have been distributed to both Hiroshima and Nagasaki storage facilities.

Immunology Studies Publications

RERF Reports (RR)

◆ Cologne JB, Preston DL, Imai K, Misumi M, Yoshida K, Hayashi T, Nakachi K: Conventional case-cohort design and analysis for studies of interaction. *Int J Epidemiol* 2012 (August); 41(4):1174–86. (RR 19-11) © The Author 2012 (This abstract was reprinted by permission of Oxford University Press.)

[Abstract] Background: The case-cohort study design has received significant methodological attention in the statistical and epidemiological literature but has not been used as widely as other cohort-based sampling designs, such as the nested case-control design. Despite its efficiency and practicality for a wide range of epidemiological study purposes, researchers may not yet be aware of the fact that the design can be analysed using standard software with only minor adjustments. Furthermore, although the large number of options for design and analysis of case-cohort studies may be daunting, they can be reduced to a few simple recommendations. **Methods:** We review conventional methods for the design and analysis of case-cohort studies and describe empirical comparisons based on a study of radiation,

gene polymorphisms and cancer in the Japanese atomic bomb survivor cohort. **Results:** Stratified, as opposed to simple, random subcohort selection is recommended, especially for studies of gene-environment interaction, which are notorious for lacking statistical power. Methods based on the score-unbiased exact pseudo-likelihood (or its analogue with stratified case-cohort data) are recommended for use in conjunction with the asymptotic variance estimator. **Conclusions:** We present an example of how to implement case-cohort analysis methods using SPSS, a popular statistical package that lacks some of the features necessary to directly adapt and implement published methods based on other software platforms. We also illustrate case-control analysis using Epicure, which provides greater risk-modelling flexibility than other software. Our conclusions and recommendations should help investigators to better understand and apply the case-cohort design in epidemiological research.

◆ Hayashi T, Morishita Y, Khattree R, Misumi M, Sasaki K, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Imai K, Kusunoki Y, Nakachi K: Evaluation of systemic markers of inflammation in atomic-bomb survivors with special reference to radiation and age effects. *FASEB J* 2012 (November); 26(11):4765–73. (RR 10-12) © FASEB (This abstract was reprinted by permission of Federation of American Societies for Experimental Biology.)

[Abstract] Past exposure to atomic bomb (A-bomb) radiation has exerted various long-lasting deleterious effects on the health of survivors. Some of these effects are seen even after >60 yr. In this study, we evaluated the subclinical inflammatory status of 442 A-bomb survivors, in terms of eight inflammation-related cytokines or markers, comprised of plasma levels of reactive oxygen species (ROS), interleukin (IL)-6, tumor necrosis factor α (TNF- α), C-reactive protein (CRP), IL-4, IL-10, and immunoglobulins, and erythrocyte sedimentation rate (ESR). The effects of past radiation exposure and natural aging on these markers were individually assessed and compared. Next, to assess the biologically significant relationship between inflammation and radiation exposure or aging, which was masked by the interrelationship of those cytokines/markers, we used multivariate statistical analyses and evaluated the systemic markers of inflammation as scores being calculated by linear combinations of selected cytokines and markers. Our results indicate that a linear combination of ROS, IL-6, CRP, and ESR generated a score that was the most indicative of inflammation and revealed clear dependences on radiation dose and aging that were found to be statistically significant. The results suggest that collectively, radiation exposure, in conjunction with natural aging, may enhance the persistent inflammatory status of A-bomb survivors.

◆ Imai K, Hayashi T, Yamaoka M, Kajimura J, Yoshida K, Kusunoki Y, Nakachi K: Effects of *NKG2D* haplotypes on the cell-surface expression of *NKG2D* protein on natural killer and CD8 T cells of peripheral blood among atomic-bomb survivors. *Hum Immunol* 2012 (June); 73(6):686–91. (RR 21-11) © American Society for Histocompatibility and Immunogenetics (This abstract was reprinted by permission of Elsevier)

[Abstract] *NKG2D* is a primary activating receptor that

triggers cell-mediated cytotoxicity in NK cells against tumor and virus-infected cells. We previously identified the *NKG2D* haplotypes in the natural killer gene complex region on chromosome 12p. Two major haplotype alleles, LNK1 and HNK1, were closely related to low and high natural cytotoxic activity phenotypes, respectively. Furthermore, the haplotype of HNK1/HNK1 has revealed a decreased risk of cancer compared with LNK1/LNK1. In the present study, using flow cytometry, we evaluated the functional effects of *NKG2D* haplotypes and five htSNPs in terms of the cell-surface expression of *NKG2D* protein on NK and CD8 T cells of peripheral blood among 732 atomic-bomb survivors. *NKG2D* expression on NK cells showed significant increases, in the order of LNK1/LNK1, LNK1/HNK1 and HNK1/HNK1 haplotypes (p for trend = 0.003), or with major homozygous, heterozygous, and minor homozygous genotypes for individual htSNPs (p for trend = 0.02–0.003). The same trend was observed for *NKG2D* expression on CD8 T cells. Our findings indicate that the *NKG2D* haplotypes are associated with the expression levels of *NKG2D* protein on NK and CD8 T cells, resulting in inter-individual variations in human cytotoxic response.

Other Journal Publications

◆ Kusunoki Y, Yoshida K, Kubo Y, Yamaoka M, Kajimura J, Hayashi T, Nakashima E, Ohishi W, Fujiwara S, Hakoda M, Akahoshi M: Effects of atomic-bomb radiation on human immune responses. Report 26: Dose-dependent increases in percentages of both Th1 and Th2 cells in the peripheral blood lymphocyte population. *Nagasaki Igakkai Zasshi* [Nagasaki Med J] 2012 (September); 87(Special Issue): 265–8. (Proceedings of the 53rd Late A-bomb Effects Research Meeting, 2012) (Japanese)

◆ Wang C, Nakamura S, Oshima M, Mochizuki-Kashio M, Nakajima-Takagi Y, Osawa M, Kusunoki Y, Kyoizumi S, Imai K, Nakachi K, Iwama A: Compromised hematopoiesis and increased DNA damage following non-lethal ionizing irradiation of a human hematopoietic system reconstituted in immunodeficient mice. *Int J Radiat Biol* 2013 (February); 89(2):132–7.

Manuscripts in Press

✂ Kyoizumi S, Kubo Y, Kajimura J, Yoshida K, Imai K, Hayashi T, Nakachi K, Young LF, Moore MA, van den Brink MRM, Kusunoki Y: Age-associated changes in the differentiation potentials of human circulating hematopoietic progenitors to T- and NK-lineage cells. *J Immunol*.

✂ Yoshida K, Kusunoki Y, Cologne JB, Kyoizumi S, Maki M, Nakachi K, Hayashi T: Radiation dose-response of *glycophorin A* somatic mutation in erythrocytes associated with gene polymorphisms of *p53 binding protein 1*. *Mutat Res-Gen Tox En*.

Immunology Studies Oral Presentations

❖ Kusunoki Y, Yoshida K, Kubo Y, Yamaoka M, Kajimura J, Hayashi T, Nakashima E, Ohishi W, Fujiwara S, Hakoda M, Akahoshi M: Effects of atomic-bomb radiation on human immune responses. Report 26: Dose-dependent increases in percentages of both Th1 and Th2 cells in the peripheral blood lymphocyte population. 53rd Late A-bomb Effects

Research Meeting, 3 June 2012,

❖ Cologne JB, Misumi M, Imai K, Preston DL, Yoshida K, Hayashi T, Nakachi K. The case-cohort design and studies of gene-environment Interaction with cancer. 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima

❖ Hayashi T. Molecular epidemiology of radiation-related cancer. 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima

❖ Hayashi T, Ito R, Cologne JB, Hayashi I, Imai K, Yoshida K, Kajimura J, Kyoizumi S, Ohishi W, Akahoshi M, Kusunoki Y, Nakachi K. Effects of *IL10* haplotype and atomic-bomb radiation exposure on gastric cancer risk. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai

❖ Hayashi T, Ohishi W, Yoshida K, Imai K, Hayashi I, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Immunogenetic factors for hepatitis virus infection and hepatocellular carcinoma observed in A-bomb survivor cohort. 21st Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics, 15–17 September 2012, Tokyo

❖ Hayashi T, Imai K, Kyoizumi S, Kusunoki Y, Nakachi K. Estimation of colorectal cancer risks in atomic-bomb survivors based on *CD14* and *IL18* gene polymorphisms and radiation exposure dose. 71st Annual Meeting of the Japanese Cancer Association, 19–21 September 2012, Sapporo

❖ Ohishi W, Fujiwara S, Chayama K. Study of viral hepatitis in a longitudinal cohort of A-bomb survivors. 16th Annual Meeting of the Japan Society of Hepatology, 10–11 October 2012, Kobe (related to *Special Clinical Studies*)

❖ Kyoizumi S, Kubo Y, Kajimura J, Yoshida K, Imai K, Hayashi T, van den Brink MRM, Nakachi K, Kusunoki Y. Age-associated shift of lymphoid potential from T to NK lineage in human hematopoietic progenitors. 74th Annual Meeting of the Japanese Society of Hematology, 19–21 October 2012, Kyoto

❖ Wang C, Nakamura S, Kusunoki Y, Kyoizumi S, Imai K, Nakachi K, Iwama A. Compromised hematopoiesis and increased DNA damage following non-lethal ionizing radiation of a human hematopoietic system reconstituted in immunodeficient mice. 74th Annual Meeting of the Japanese Society of Hematology, 19–21 October 2012, Kyoto

❖ Hayashi T, Yoshida K, Ohishi W, Kyoizumi S, Kusunoki Y. Evaluation of inflammation status in atomic-bomb survivors with special reference to radiation and age effects. 41st Annual Meeting of the Japanese Society for Immunology, 5–7 December 2012, Kobe

❖ Kyoizumi S, Yoshida K, Hayashi T, van den Brink MRM, Moore MA, Kusunoki Y. Evaluation of T- and NK-cell potentials of human circulating hematopoietic progenitors. 41st Annual Meeting of the Japanese Society for Immunology, 5–7 December 2012, Kobe

❖ Nakachi K. The impact of radiation exposure on the aging human immune system. 25th International Symposium “Radiation and Cancer,” 6–8 December 2012, Tokyo (related to *Cell Biology Studies*)

Research Protocols 3-11, 3-10, 2-10, 6-08, 4-08, 3-05, 2-05, 1-05, 5-00, 3-00, 2-99, 9-92, 5-92, 3-89, 4-85, A7-12, A4-12, A2-12, A1-12, A6-11, A4-11, A1-10, A5-09, A14-08, A13-08, A10-08, 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, Cologne JB (S), Ohshita T, Miyachi T, Matsumoto M, Tsujino A, Mimori Y, Sasaki H, Nakamura S, Krull KR, Fujiwara S

Objectives: Objectives, are (1) to examine associations between radiation exposure at ages 0–12 or *in utero* and neurocognitive function in late life as assessed by neuropsychological examinations, (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 and the occurrence of dementia with advancing age.

Background and Significance: The atomic bomb study and various studies of childhood radiotherapy have shown that the brain is susceptible to radiation damage *in utero* and in early childhood. The proposed study is a unique opportunity to examine the prenatal and early childhood effects of radiation exposure on cognitive function as it appears more than 65 years later.

Study Methods: Study subjects are survivors exposed at ages 0–12 or *in utero*. We will evaluate neurocognitive function for about 1,050 subjects in Hiroshima and Nagasaki using the Cognitive Abilities Screening Instrument (CASI) and the Childhood Cancer Survivors Study (CCSS) Neurocognitive Questionnaire (NCQ) during the period 2011–2015. For the CASI, assessment is based on face-to-face interviews by trained nurse technicians during routine health examinations in the AHS. The self-administered NCQ will be conducted by mail survey.

Study Progress: Evaluation of neurocognitive function using CASI and NCQ was started in 2011. About 550 subjects have been investigated using CASI. About 1,200 subjects have answered the NCQ.

Results and Conclusions: None yet.

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

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

Objectives: This addendum study proposes to investigate unanswered questions in the previous ophthalmologic study based on RP 3-00, (1) whether or not radiation-induced cataracts progress with time and (2) whether or not there is a dose response when cataracts are assessed by a radiation-specific classification system (the Merriam-Focht system).

Background and Significance: The ophthalmologic study conducted from 2000 to 2002 based on RP 3-00 had

revealed a statistically significant dose response with posterior subcapsular and cortical cataracts. The study also suggested a low or no dose threshold. However, important research questions still remain unanswered.

Study Methods: (1) Subjects will be those who were age 13 or less at the time of the bombings. (2) The Lens Opacity Classification System II and the Merriam-Focht system will be used for grading by the ophthalmologists. (3) A variety of potential confounding factors will be incorporated in the analysis. (4) Digital computer images of the lens and retina will be stored.

Study Progress: The ophthalmologic examinations started in August 2010 in Hiroshima and Nagasaki; 418 participants in Hiroshima and 231 participants in Nagasaki were examined by an ophthalmologist as of October 2012.

Results and Conclusions: None yet. Results expected in 2013.

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)

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

Objectives: We will investigate if retinal arteriolosclerosis is associated with radiation dose and is involved as an intermediate risk factor in radiation-associated glaucoma.

Background: Retinal vessel caliber is a well-established non-invasive marker of microvascular disorders, which may contribute to the initiation and/or progression of radiation-induced cardiovascular diseases. Furthermore, alterations of ocular perfusion could cause ischemia and poor perfusion of the optic disc would be a major cause of glaucomatous damage. Preliminary analyses of the glaucoma study during 2006–2008 suggested a relationship between radiation and an increased prevalence of normal-tension glaucoma among A-bomb survivors. To investigate the pathological background of glaucoma, we planned to calculate retinal vessel diameters, which are possibly involved in glaucoma genesis through eye-circulation impediment. In addition, we will be able to investigate the relationship between radiation and age-related macular degeneration, which will be assessed using the same retinal images.

Study Methods: This is a cross-sectional study of AHS subjects for whom retinal photography was performed during 2006–2008. Vessel calibers and age-related macular degeneration were assessed from the digitized retinal images at the University of Melbourne using a computer-assisted program.

Study Progress: Well-trained graders at the Melbourne (Australia) ophthalmology center have calculated the width of retinal arterial and venous vessels and graded the macular degeneration separately using a semi-automated computer program. All grading was performed blinded with respect to radiation dose. We conducted the analysis and have submitted a manuscript about the smoking effects on retinal

vessel calibers. Analyses of retinal vessel caliber and radiation dose are underway. The grading of macular degeneration has been completed and analysis has been started.

Results: We will continue analyses to examine possible associations of radiation exposure with vessel diameters and the endpoints of glaucoma and macular degeneration.

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

Ohishi W (CH), Tatsukawa Y (CH), Ueda K (CH), Fujiwara S, Nakashima E (S), Kohata M, Yamada M (CH), Ozasa K (EH), Tsuge M, Chayama K

Objectives: The hypothesis behind this study is that radiation exposure may accelerate the severity of liver fibrosis irrespective of hepatitis virus infection. The objective is to determine whether A-bomb radiation exposure has increased liver stiffness, which serves as a marker of liver fibrosis severity, and to investigate the possibility that liver fibrosis is involved in the development of atherosclerotic diseases by inducing insulin resistance.

Background and Significance: Liver fibrosis sometimes progresses into liver cirrhosis and hepatocellular carcinoma (HCC), among chronic type B or C liver disease and nonalcoholic steatohepatitis cases. The RERF data have shown that both chronic liver disease and liver cirrhosis are related to radiation dose. In the LSS and AHS populations, radiation effects on the incidence of atherosclerotic diseases, such as hypertension and cardiovascular disease, have also been observed.

Study Methods: We will examine the relationship between liver stiffness and radiation dose, in order to determine whether this is a plausible pathway by which radiation exposure is involved in increased chronic hepatitis and liver cirrhosis. We will also examine whether an increase in liver fibrosis is involved, through insulin resistance, in the development of atherosclerotic diseases, in order to elucidate mechanisms of radiation effects underlying these diseases.

Study Progress: During the period from November 2008 to November 2012, we measured liver stiffness with the elastometer for about 2,900 AHS participants. We completed measurements of liver stiffness for younger A-bomb survivors exposed before age 10 among the AHS and started data cleaning after creation of the data set, which also includes blood cytokine levels and other clinical examination and information data. We measured blood cytokine levels with the ELISA method or the multiplex bead array assay for 2,900 survivors.

Results and Conclusions: None yet. Results expected in 2014.

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

Hida A (CN), Tatsukawa Y (CH), Neriishi K, Blakely EA, Chang P, Nakashima E (S), Ohishi W (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

Objectives: Little is known about the mechanisms by which radiation causes cataracts. Samples of cataract tissue from

radiation-exposed study subjects will provide an opportunity to study mechanisms and pathways in more detail. The goal of this preliminary project is to confirm the adequacy of the storage method for cataract tissues of the AHS participants who undergo a cataract operation, and to collect and store the tissue for future analyses.

Background and Significance: Our recent study found a radiation effect with an odds ratio at 1 Gy of 1.39 (95% confidence interval 1.24, 1.55) for the prevalence of cataract operations. Cataract extractions become more common as the AHS participants age. The potential to evaluate the molecular biology from stored lens tissues will add significantly to our knowledge about radiation-induced cataract.

Study Methods: 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 conducted.

Study Progress: The program of collection and storage of lens tissue is continuing. Fifty-four samples in Hiroshima and 17 samples in Nagasaki have been collected and stored as of March 2012. Among these, opacities in lenses had previously been detected at RERF for 13 cases. We tried to extract RNA and DNA from the lens epithelial cells attached to the anterior capsule. A member of the study team has met with several leading ophthalmologic researchers to develop initial plans regarding the biological questions that can be addressed by measurements made on these biosamples.

Results and Conclusions: None yet. Results expected in 2013.

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

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

Objectives: To explore effects of inflammation levels on radiation risk for cancer development.

Background and Significance: Experimental and epidemiological studies report a relationship between inflammation and cancer. Because A-bomb survivors have radiation dose-dependent increases in inflammatory biomarkers, we are investigating the relationship between selected biomarkers and cancer incidence among 12,870 AHS participants followed from 1965 to 1999 and their possible role in radiation risk for cancer.

Study Methods: A number of inflammation biomarkers have been measured in the AHS cohort. Since associations between radiation, biomarkers and cancer outcomes are complex, we are using several statistical analysis approaches to characterize them.

Study Progress: Initial analyses of the joint effects of white blood cell (WBC) and radiation or smoking on cancer incidence using a causal model were completed and a manuscript has been written.

Results and Conclusions: Longitudinal trends in WBC counts over time are elevated among subjects exposed to ≥ 2 Gy radiation (result published in *Journal of Radiation Research* in 2010). There is a significant mediation effect by the longitudinal WBC count upon the radiation risk for all solid cancer, with the proportion of total radiation effect

attributed to mediation by WBC being about 7%. Whether mediation by WBC of the radiation risk depends on subtype of cancer could not be determined for many subtypes, but the mediation proportion for lung cancer was much higher (27%). A manuscript has been internally approved and is currently in the journal review process.

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

Ohishi W (CH), Takahashi I (CH), Cologne JB (S), Akahoshi M (CN), Fujiwara 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. Specifically, it is hypothesized that if there was survival bias due to the early sequelae of the bombing, then genetic profiles which are associated with the degree of inflammatory responses to biologic stressors would likely differ among high-dose survivors vs. high-dose non-survivors, which could cause the survivors to differ from (virtually) unexposed controls in either the frequency of relevant genes or in the genotype Hardy-Weinberg Equilibrium (HWE). Secondly, since the targeted inflammatory genes may also be implicated in cardiovascular disease, we will also examine whether they and radiation dose are jointly related to increased risk of cardiovascular disease.

Background and Significance: Since the Life Span Study (LSS) and Adult Health Study (AHS) cohort members had to have survived until 1950 and 1958, respectively, a “healthy survivor effect” may have been operative with respect to noncancer mortality among the high-dose survivors. The hypothesis is that genetic polymorphisms controlling responsiveness to external stresses can alter survival rates following acute radiation exposures and also increase cardiovascular disease in later life. Specifically, candidate genetic polymorphisms in the *LTA* and *TRL2* genes were identified as important stress-responsiveness and chronic inflammation genes.

Study Methods: A case-control study among younger survivors who participated in the first health examination in 1958–1959 and were exposed to at least 1 Gy of radiation, and their sex-, age-, and city-matched controls exposed distally to less than 5 mGy will be conducted. We will analyze selected *LTA* and *TRL2* genetic polymorphisms to determine if there is dose-related evidence for differences in their frequency or in HWE. We will also assess the joint association of radiation and the *LTA* and *TRL2* genotypes to see if the radiation effect is modified by genotype.

Study Progress: The genotyping (*LTA* and *TRL2* genes) was completed, and the relationship between the *LTA* and *TRL2* genotype frequency and A-bomb radiation exposure is being examined. The preliminary results showed that both *LTA* and *TRL2* genotype frequencies are not significantly different between proximal and distal survivors. We will further examine whether the HWE differs by radiation dose, and whether the genotypes modify the radiation-car-

diovascular disease associations.

Results and Conclusions: None yet. Results expected in 2014.

RP 1-05 Glaucoma study in atomic bomb survivors

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

Objectives: To determine the relationship between radiation dose and prevalence of glaucoma.

Background and Significance: Although it is well known clinically that acute, high radiation exposure can induce glaucoma, the relationship between glaucoma and radiation exposure within the general population at moderate doses, such as in the AHS cohort, is unclear.

Study Methods: We conducted comprehensive ophthalmologic examinations, including screening examinations with intraocular pressure, retinal images and a visual field examination, with further detailed examinations for potential cases during 2006–2008.

Study Progress: Among 1,589 subjects with known radiation dose (mean age 74.3) we detected 284 (17.9%) cases of glaucoma overall, most of whom presented with normal tension glaucoma. Based on suggestions of a Glaucoma Study Group various aspects of the study, including possible non-participation bias, were analyzed and a manuscript was submitted to an international journal.

Results and Conclusions: The analyses using the generalized estimating equation method, with adjustment for sex, age, city, cataract surgery, and diabetes mellitus, revealed an elevated odds ratio in the case of normal tension glaucoma, but the findings need to be interpreted cautiously given possible biases associated with the high, non-random non-participation rates.

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, Seto S

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

Background and Significance: 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, which has prompted us to search for sex-related co-factors.

Study Methods: We identified 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 ascertained the sudden death cases to evaluate the prognosis of Brugada type ECG cases. We also evaluated the association between Brugada type ECG and prostatic cancer, which is related to testosterone.

Study Progress: 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.

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

RP 3-00 Ophthalmologic study of atomic bomb survivors

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

Objectives: For a number of decades the radiation risk-assessment community had long regarded the induction of opacities by radiation as having a dose threshold above 1 Gy, though that threshold level was recently lowered, in large part because of the AHS A-bomb data. The goal of this study is to obtain more precise estimates of the prevalence of radiation cataracts across a range of lower doses and examine modification of radiation effects by other factors.

Background and Significance: The mechanism(s) of radiation cataractogenesis is still unknown. The A-bomb survivor cohort is unique and valuable to provide insights on the mechanism(s) and bases for radiation safety regulation. Stored lens images collected during 2000–2002 are useful for many purposes, inter- and intra-cohort comparison and others.

Study Methods: (1) 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. (2) Stored lens images are to be used to study longitudinal opacity progression.

Study Progress: Using stored lens images, (1) we have conducted the analyses regarding a lens opacity re-evaluation with the Merriam-Focht method, and (2) a manuscript on misclassification of cataract surgery has been submitted to an international journal. A manuscript on cataract surgery incidence has been published in *Radiology*.

Results and Conclusions: (1) The results of an opacity re-evaluation with the Merriam-Focht method indicate that the dose-response slopes for A-bomb survivors and Chernobyl clean-up workers are almost identical. (2) Under certain assumptions, statistical modeling suggested that up to 40% of survivors who had clinically-significant cataracts may not have had cataract surgery, which however did not change the dose response for cataract surgery.

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, Ohishi W (CH), Yamada M (CH), Nakashima E (S), Okubo M, Ashizawa K, Sera N (CN)

Objectives: To investigate whether there are positive associations between radiation dose and thyroid diseases in the AHS cohort, and to examine how frequently thyroid cancer has developed among subjects with thyroid nodules

detected in an earlier study (1984–1987).

Background and Significance: An earlier study of thyroid disease in Nagasaki AHS cohort in 1984–1987 showed an approximately linear association between thyroid radiation dose and the prevalence of thyroid nodules and suggested an association between autoimmune hypothyroidism and radiation dose (*JAMA* 1994; 272:364). The current thyroid study is conducted in both Hiroshima and Nagasaki AHS cohort members to clarify the radiation dose response for thyroid diseases. A second objective is to examine whether thyroid cancer develops frequently among irradiated subjects with thyroid nodules detected in the previous study (1984–1987). If they do, this would impact guidelines for clinicians regarding follow-up of nodules and incidental tumors.

Study Methods: Thyroid examinations are performed (blinded as to radiation dose) in AHS cohort members and dose responses for thyroid disorders are analyzed. For the second objective, incident thyroid cancer is ascertained among those with thyroid nodules in 1984–1987.

Study Progress: We performed thyroid examinations in AHS cohort members between 2000 and 2003. We are continuing the follow-up of AHS subjects for additional thyroid cancers. We conducted thyroid examinations in AHS subjects exposed at younger ages between 2008 and 2010 (RP 3-07) for further study of radiation effects on thyroid diseases and are making final thyroid diagnoses of the subjects.

Results and Conclusions: We found that both malignant thyroid tumors and benign nodules were increased with radiation dose and the relationships were significantly higher in those exposed at younger ages. On the other hand, autoimmune hypothyroidism and Graves' disease were not associated with radiation dose (*JAMA* 2006; 295(9):1011–22). No significant dose responses for thyroid diseases were observed among those exposed *in utero* (*J Clin Endocrinol Metab* 2008; 93:1641–8), though that may reflect lack of statistical power to detect effects. We more frequently detected cancer in subjects with solid thyroid nodules than in nodule-free controls (*J Clin Endocrinol Metab* 2005; 90:5009–14).

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

Objectives: The hypothesis behind this study is that radiation may increase the incidence of hepatocellular carcinoma (HCC) either by increasing the rates of chronic hepatitis B (HBV) and C (HCV) virus infection, or by accelerating the disease progression after hepatitis virus infection. The objective of the study is to investigate the relationship between radiation dose and the natural history of HBV or HCV infection among AHS cohort.

Background and Significance: Our previous studies demonstrated that the prevalence of hepatitis HBsAg increased with radiation dose among the AHS. The percent among subjects who were unable to clear the virus increased significantly with radiation dose among those receiving

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.

Study Methods: We will examine (1) the relation between radiation dose and HBV activity ascertained by measuring hepatitis B e-antigen (HBeAg) and HBV DNA, and the HBeAg or HBsAg seroconversion rates, (2) the relation between radiation dose and the natural history of chronic type B or C liver diseases, and (3) the effects of clinicopathological features, immunogenetic background, and radiation dose on the course following hepatitis virus infection among AHS cohort.

Study Progress: The relationship between *NKG2D* genotypes (RP 4-04) and radiation exposure on hepatitis virus infection status and HCC were assessed preliminarily in collaboration with the Department of Radiobiology/Molecular Epidemiology.

Results and Conclusions: Subjects with persistent HCV infection showed Th1-dominant immunological features. Increased Th1 cell percentages were significantly associated with accelerated progression of liver fibrosis, while Tc1 and NK cell percentages were inversely associated with fibrosis progression (*Hum Immunol* 2011; 72:821–6).

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

Yamada M (CH), Mimori Y, Cologne JB (S), Fujiwara S, Sasaki H, Akahoshi M (CN), Matsumoto M, Kasagi F, White LR

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

Background and Significance: This study investigates the hypothesis that the effects of ionizing radiation on the mature central nervous system could possibly 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 Methods: 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). A total of 2,286 dementia-free subjects at baseline examination have been followed up to assess dementia incidence and results were published.

Study Progress: The database of the CASI longitudinal examinations has been restructured. Analyses as to “Shape of cognition trajectories in late adulthood, especially non-demented subjects” and “Risk of decline of cognitive function” are planned in cooperation with the Department of Statistics.

Results and Conclusions: No association was found between previous radiation exposure and cognitive impairment and/or development of dementia among subjects exposed at ≥ 13 years of age. Additional longitudinal analyses regarding cognitive decline and risk factor of dementia have been initiated.

RP 3-89 Osteoporosis in Hiroshima atomic-bomb survivors

Fujiwara S, Takahashi I (CH), Ohishi W (CH), Masunari N, Furukawa K (S), Nakamura T, Yoshimura N, 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 after adjusting for age, weight, and age at menopause. We are utilizing accumulated data on BMD and fracture for national and international collaborative studies that provide new insights regarding BMD and guidelines for health maintenance of atomic bomb survivors and others.

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 and have published several papers.

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 (*Osteoporos Int* 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 (Addendum to Research Plan TR 12-71)

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

Objectives: 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 to the entire AHS cohort.

Background and Significance: 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, stroke, calcification of the aortic arch, retinal

arteriosclerosis, isolated systolic hypertension, and abnormal pulse wave velocity.

Study Methods: 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.

Study Progress: An RP to assess atherosclerosis-associated cytokines was started in 2011 in addition to measurement of central blood pressure, augmentation index, brachial-ankle pulse wave velocity, and ankle brachial index which began in 2010.

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

RP-A7-12 Serum TSH within the reference range and the risk of coronary heart disease (Addendum to RP-A10-08)

Åsvold BO, Imaizumi M (CN)

Objectives: To study the associations of serum thyrotropin (TSH) concentrations within the reference range with 1) the risk of a first-time coronary heart disease event and specifically, the risk of first-time myocardial infarction, 2) survival after myocardial infarction, 3) the risk of death from coronary heart disease.

Background and Significance: We previously reported that subclinical hypothyroidism, defined as elevated serum TSH levels with normal thyroxine concentrations, was associated with an increased risk of coronary heart disease events and mortality by using combined data from the Thyroid Studies Collaboration, the multicenter and international collaboration including RERF. Recently, some people with TSH in the upper part of the reference range have an early stage of hypothyroidism. However, an association between TSH within the reference range and coronary heart disease has been unknown. By using combined data from the Thyroid Studies Collaboration, we will study the associations of TSH within the reference range with the risk of coronary heart disease, the risk of myocardial infarction, survival from myocardial infarction, and the risk of death from coronary heart disease.

Study Methods: Study cohorts include all study populations participating the Thyroid Studies Collaboration. We will analyze individuals with TSH within the reference range. For each cohort, we will use Cox regression analysis to estimate hazard ratios for a first-time coronary heart disease event, first-time myocardial infarction, and death from coronary heart disease by categories of TSH within the reference range, using the lower part of the TSH reference range (0.45–1.49 mU/L) as reference group. We will use random-effects meta-analysis to obtain pooled estimates, analogous to the method used in two papers from the Thyroid Studies Collaboration (*JAMA* 2010; 304:1365 and *Arch Intern Med* 2012; 172:799).

Study Progress: We are performing analyses.

Results and Conclusions: Not yet. Results expected in 2013.

RP-A4-12 MRI-based study of importance of diagnosis of fatty infiltration of the salivary glands among patients with xerostomia

Takagi Y, Hida A (CN), Nakamura H, Sumi M, Akahoshi M (CN), Nakamura T

Objectives: Using MRI imaging, we evaluated the salivary glands of patients with xerostomia to study status and incidence of “fatty salivary glands” and the condition’s casual relationship with glandular dysfunction.

Background and Significance: Fatty degeneration and fatty deposition are known to occur in salivary gland tissue of patients with Sjögren’s syndrome (SS) and dyslipidemia (DL), a situation that can be confirmed by MRI. Although the association between “fatty salivary glands” and glandular dysfunction has been suggested, the former’s clinical significance and mechanism are not fully understood.

Study Methods: Among 235 subjects who were examined and underwent MRI examination at the Xerostomia Clinic of the Nagasaki University Hospital, 155 subjects were referred from RERF between 2003 and 2005 based on a pilot study of AHS sicca syndrome. The subjects comprised 80 patients with SS, 70 patients with DL, and 85 non-SS and non-DL subjects. Salivary gland MRI imaging is evaluated retrospectively to confirm status and incidence of fatty salivary glands and to examine the condition’s causal relationship with glandular dysfunction.

Study Progress: The analysis of MRI imaging of 235 subjects was finished and a paper has been written.

Results and Conclusions: The salivary flow rates of SS patients were significantly correlated with the percentages of fat area in the glands. The salivary flow rates of non-SS patients were not correlated with the percentages of fatty area in the glands. Further, salivary flow rates of DL and non-DL patients were similar, irrespective of the presence or absence of fat infiltration in these patients.

RP-A2-12 Weight fluctuation and cancer and cardiovascular disease incidence and mortality in Japanese

Nanri A, Mizoue T, Sera N (CN), Takahashi I (CH), Soda M (EN), Suyama A, Ozasa K (EH), Cologne JB (S), Araki Y, Hsu WL (S), Cullings HM (S), Ohishi W (CH), Akahoshi M (CN)

Objectives: We identify patterns of weight fluctuation and examine their association with subsequent morbidity and mortality of cancer and cardiovascular disease in a Japanese population.

Background and Significance: Obesity and underweight at a single point in time, and weight gain and loss, have been reported to be associated with increased mortality. Some studies have also found an association between weight fluctuation and mortality. However, measures of weight fluctuation in previous studies have had limited ability to capture weight fluctuation patterns in terms of the frequency and magnitude of weight change.

Study Methods: In this study, we first calculate a classical measure of weight fluctuation and, to address the above issue, further develop innovative measures that facilitate

examining the relationship of longitudinal patterns in weight change to morbidity and mortality. We will prospectively examine the association of the new indicators of weight fluctuation with morbidity and mortality from all causes, cancer, and cardiovascular disease using Cox regression. Analyses will be based on 5,790 subjects in the Adult Health Study (AHS) cohort who were aged 20 to 49 years old at baseline (1958) and had health examination seven times or more between baseline and start of follow-up 20 years later (1978). The analysis should provide improved estimates of the risk of weight fluctuation based on enhanced conceptual and statistical approaches compared with those used in previous studies.

Study Progress: We have started to complete the data set since July 2012.

Results and Conclusions: None yet. Results expected in 2016.

RP-A1-12 Study for the epidemiological and genetic basis of progressive cardiac conduction defect

Makita N, Akahoshi M (CN), Haruta D, Maemura K, Ohishi W (CH), Nakashima E (S)

Objectives: We will focus on progressive cardiac conduction defect (PCCD), which is a hereditary lethal arrhythmia.

Background and Significance: PCCD is a hereditary lethal arrhythmia characterized by electrocardiographic findings of atrioventricular block and bundle branch block caused by progressive degenerative fibrosis of cardiac conduction system. Sudden death and pacemaker implantation are outcomes of PCCD and three genes have been reported as responsible for PCCD. We will determine the possible PCCD cases who progressed to sudden death or pacemaker implantation from bundle branch block in AHS and conduct gene analysis in these possible PCCD cases.

Study Methods: Among 16,170 individuals who underwent health examinations in the Adult Health Study in Nagasaki and Hiroshima between 1967 and 2010, we will extract those who were diagnosed with right bundle branch block (RBBB; 828 cases). They will be categorized into two groups: a group (non-PCCD) who did not show progression of the bundle branch block, and a case group (PCCD) that progressed into sick sinus syndrome (SSS) or complete atrioventricular block (AVB). Latter group includes individuals who have undergone pacemaker implantation. Endpoints of the study are pacemaker implantation (32 cases).

At Nagasaki University, genomic DNA will be extracted from peripheral lymphocytes of subjects in the case group, and the genetic screening will be conducted on the following genes: connexin genes, which modulate the electrical connection between cardiac cells (connexin 40, 43, 45), Na channels (*SCN10A*, *SCN4B*), which have been raised as candidate genes affecting cardiac conduction in genome-wide association analysis, and the cytoskeletal protein lamin, which plays a role in maintenance of structure of cellular nucleus and modulation of transcription. Exons of the respective genes will be amplified by the PCR method, and the base sequences will be analyzed with an ABI 3130 sequencer.

Study Progress: We detected 561 incident RBBB cases and selected 1,120 age- and sex- matched controls among

16,170 AHS subjects. We found 16 and 6 pacemaker implanted cases due to SSS or AVB in incident-RBBB cases and age- and sex- matched controls.

Results and Conclusions: None yet. Results expected in 2014.

RP-A6-11 Study on the genetic background of short QT

Makita N, Akahoshi M (CN), Haruta D, Maemura K, Ohishi W (CH), Fujiwara S

Objectives: Congenital short QT syndrome (SQT) is a genetic disease characterized by sudden death due to ventricular arrhythmia, and five cardiac ion channel genes have been reported as responsible genes. Because only several dozen patients with short QT have been reported, we will conduct gene analysis in two subjects with short QT (prevalence was 0.01%) who were identified by Dr. Moriya in the RERF AHS.

Background and Significance: SQT is a genetic arrhythmia characterized by shortened electrocardiographic QT interval (QTc <350 msec) and sudden death. In 2007, Moriya et al. at the Nagasaki Laboratory of RERF examined electrocardiograms of 19,153 subjects in a follow-up program, and observed short QT (QTc <350 msec) in two of them. This study is aimed at determining whether there are SQT gene abnormalities in the two subjects who exhibited SQT in the RERF follow-up program.

Study Methods: Blood samples are available from both SQT cases. The gene analysis will be conducted at the Department of Molecular Physiology of Nagasaki University. The genes to be analyzed are those for three K channels (*KCNH2*, *KCNQ1*, and *KCNJ2*) and two Ca channels (*CACNA1C* and *CACNB2*) that have been reported as SQT genes. We will amplify the exons of the respective genes by PCR technique and read the DNA sequences using an ABI 3130 capillary sequencer. When gene abnormalities are observed, we will prepare mutant cDNA and transfect this DNA into CHO cell culture in order to analyze electrophysiological characteristics of mutant channels by the whole-cell patch clamp method to refine the functional abnormalities that tend to shorten the action potential of cardiomyocytes.

Study Progress: One case is still alive and another case was deceased. The living case will visit RERF during FY 2013 or 2014.

Results and Conclusions: None yet. Results expected in 2015.

RP-A4-11 Role of visceral fat and its effects in the association between radiation dose and cardiovascular disease

Sera N (CN), Hsu WL (S), Nakashima E (S), Carter RL, Hida A (CN), Imaizumi M (CN), Cullings HM (S), Akahoshi M (CN)

Objectives: We plan to investigate the causal pathways and associations among atomic bomb (A-bomb) radiation, visceral fat accumulation and its sequelae (fatty liver, hypertension, hyperlipidemia, type 2 diabetes mellitus), and cardiovascular disease (CVD).

Background and Significance: It has been reported that A-bomb radiation exposure increases the risk of CVD. In addition, A-bomb radiation is related to fatty liver, hyper-

tension, abnormal lipid profiles, impaired glucose tolerance, and inflammation, which are all related to visceral fat accumulation. Free fatty acids (FFAs) and several adipokines (inflammatory cytokines, adiponectin, angiotensinogen, and plasminogen activator inhibitor-1 [PAI-1]) are secreted from visceral fat (adipocytes and macrophages in fat tissue). FFAs and adipokines, along with visceral fat accumulation, may contribute to the mechanism(s) of radiation-induced CVD. These results indicate that visceral fat accumulation and metabolic and/or inflammatory parameters relating to FFAs, and adipokines, may be part of the causal pathway that explains the association between A-bomb radiation and CVD.

Study Methods: We examined 1,366 Nagasaki Adult Health Study (AHS) subjects (521 male, 845 female) from 2004 through 2007 and collected (1) the data of surrogate markers of visceral fat accumulation and atherosclerosis, and (2) metabolic or inflammatory data relating to FFAs and adipokines. We also identified the cases of hypertension, type 2 diabetes, hyperlipidemia, metabolic syndrome, angina pectoris, myocardial infarction, and stroke, based on standard diagnostic criteria, after taking consideration of medication history. Using this data set, we plan to investigate the potential causal pathways and associations among A-bomb radiation, visceral fat accumulation and its sequels (fatty liver, hypertension, hyperlipidemia, type 2 diabetes), and CVD. The mediating effects of visceral fat accumulation on the radiation-CVD association will be investigated using the conceptual model: radiation + visceral fat accumulation → inflammation → metabolic function → atherosclerosis → CVD.

Study Progress: We have conducted demographic analyses and got positive association of IHD with hypertension and diabetes mellitus.

Results and Conclusions: None yet. Results expected in 2014.

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

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

Objectives: This study aim is to investigate an association between *ATM* polymorphisms and radiosensitivity differences with respect to cataract surgery prevalence among A-bomb survivors.

Background and Significance: The mechanisms of the relationship between radiation and cataract in A-bomb survivors remain unclear. We are investigating if *ATM* gene polymorphisms, which have been shown to enhance X-ray-induced cataractogenesis in animal experiments, are related to radiation-induced cataract surgery prevalence among A-bomb survivors.

Study Methods: The study is examining the prevalence of surgically-removed cataracts in the AHS in relation to radiation dose and the presence of selected *ATM* polymorphisms. The examination included 3,744 subjects during 2000 and 2001.

Study Progress: We reviewed the utility of cataract surgery as a measure for this genetic study and the appropriate analyses for radiation by gene interaction.

Results and Conclusions: None yet

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

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

Objectives: To apply a joint model to investigate how radiation and other risk factors, such as smoking, result in elevated inflammation levels and a higher risk of cataract surgery.

Background and Significance: 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.

Study Methods: This study proposes to apply a joint model in which the associations among radiation, inflammation, and incidence of cataract surgery, allowing inflammation to be a mediating variable, are estimated in a time-to-event analysis for the A-bomb survivors.

Study Progress: Analyses have been delayed due to a problem with statistical methods.

Results and Conclusions: The data obtained from 3,942 Adult Health Study participants indicate that radiation exposure is significantly associated with cataract ($P < 0.001$) and elevated inflammation ($P = 0.016$), and inflammation is a significant risk factor for cataract ($P = 0.008$). The mediating proportion via inflammation is roughly 7.2% of the total radiation effect at 1 Gy. The joint model supports the hypothesized causal pathway of radiation exposure, chronic inflammation, and cataract, although inflammation accounts for only a small fraction of the radiation effect. However, in this preliminary analysis, inflammatory diseases were not taken into account.

There is a methodological question as to whether the analysis should use longitudinal data or cross-sectional data, as the Kurume group reported that the Cox model did not work well with the data set. The prevalence analysis using M-plus software can be made for the inflammatory measurements measured during the period from 1988 to 1992 with cataract surgery data during 1986 to 2005, and a latent variable that reflects the inflammation that will be produced by factor analysis using M-plus. The indirect effect of eye radiation dose via inflammation on cataract surgery and the direct effect of eye radiation dose will be jointly estimated by M-plus, including the effect of inflammatory diseases. A formal analysis should be made before writing a manuscript.

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

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

Objectives: To examine the incidence of the early repolarization pattern (ERP) and its prognostic value in terms of mortality from unexpected death, cardiac disease mortality, and total mortality among Nagasaki AHS subjects.

Background and Significance: The majority of sudden cardiac deaths are caused by ventricular tachyarrhythmias which occur in persons with a structurally normal heart. The

ERP has been considered benign, but one experimental study reported its potential arrhythmogenicity (Gussak I. et al., *J Electrocardiol* 2000; 33:299–309), suggesting the possibility that ERP is a cause of idiopathic ventricular fibrillation leading to sudden death.

Study Methods: We reviewed all the ECG records of 5,976 AHS subjects who were examined at least once between 1958 and 2004. The Brugada-type ECG cases were excluded. We identified those who showed ERP and calculated the incidence during the follow-up period after excluding prevalent ERP cases. We assessed the cause of death using death certificates and calculated the risk of: 1) unexpected deaths including sudden death or unexplained accidental death; 2) cardiac disease mortality; and 3) total mortality in ERP cases by means of Cox proportional hazards analysis with adjustment for age, sex and underlying cardiovascular diseases.

Study Progress: We have finished analyses of the associations of ERP with unexpected death, cardiac death and total mortality.

Results and Conclusions: ERP incidence was 715 per 100,000 person-years and ERP was observed in 35.5% of unexpected death cases. ERP had an elevated risk of unexpected death (HR 1.83, 95% CI 1.12–2.97), and a decreased risk of cardiac (HR 0.75, 95% CI 0.60–0.93) and all-cause (HR 0.83, 95% CI 0.78–0.93) death. ERP has significant public health implications to prevent unexpected death.

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

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

Objectives: Ventricular premature contractions (VPC) in regular 12-lead electrocardiogram (ECG) recordings has again gained attention as a predictive variable for cardiovascular mortality. Thus, we would like to evaluate the significance of VPC in regular 12-lead ECG recordings as a risk factor for cardiovascular mortality in the general population.

Background and Significance: Since the CAST trials found no evidence for the theory that VPC suppression reduces risk, VPC has generally been ignored on a routine ECG examination. But, some recent reports have indicated that the presence of VPC in regular 12-lead ECG recordings is a significant and independent predictor of cardiovascular mortality.

Study Methods: We will identify VPC cases from AHS subjects (4,092 in Hiroshima and 2,642 in Nagasaki) who underwent a regular 12-lead ECG recording in 1990 to December 1993. We will identify subjects with VPC, and classify into three groups according to VPC morphology: (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. We will compare the basic characteristics at VPC diagnosis, and the underlying diseases between those with and without VPC and also among VPC cases with LBBB type, RBBB type, and unidentified type. Information on mortality and cause of death until December 2005 will be used to assess the prognostic significance for cardiovascular mortality between those with and without VPC. We will

conduct a Cox proportional hazards analysis to assess the prognostic significance of VPC, frequency of cardiovascular mortality according to VPC diagnosis and morphology of VPC with adjustment for age, sex, and underlying diseases.

Study Progress: To date we have reviewed only a small number of subjects, because we treated RP-A14-08 as a top priority compared to RP-A13-08.

Results and Conclusions: None yet. Results expected in 2014.

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)

Objectives: 1) To assess the relationship between subclinical thyroid dysfunction and coronary heart disease (CHD) and mortality, 2) To assess whether these relationships persist after adjusting for important potential confounders, and 3) To determine whether these relationships differ according to: age and gender, race, TSH levels, or presence or absence of prevalent cardiovascular disease.

Background and Significance: Subclinical hypothyroidism has been reported to be associated with elevated cholesterol and increased risk for atherosclerosis. We previously reported on risk for ischemic heart disease and all-cause mortality in subclinical hypothyroidism (*J Clin Endocrinol Metab* 2004; 89:3365). In this study, we found a significant association between the prevalence of ischemic heart disease and subclinical hypothyroidism and a possible increased mortality in men with subclinical hypothyroidism by using the data of 2,856 Nagasaki AHS subjects examined between 1984 and 1987. However, several reports from other cohorts on the relationship between subclinical hypothyroidism and CHD events and mortality are conflicting. Only a pooled analysis of the large cohort studies with individual participant data might confirm these associations, explore these potential differences, and clarify these conflicting data.

Study Methods: We are performing a pooled analysis of individual participants in large international cohort studies to determine whether there is an association between subclinical thyroid dysfunction and cardiovascular disease and mortality. A total of nine cohorts including the Nagasaki AHS subjects are included in the pooled analysis.

Study Progress: The 11 prospective studies including the Nagasaki AHS included 55,287 participants with 542,494 person-years of follow-up. The analyses led to a high-profile publication (Rodondi et al. [including Imaizumi M], *JAMA* 2010; 304(12):1365–74). We are evaluating the association between subclinical hypothyroidism and cardiovascular risk factors according to age based on the small-scale RP related to this RP.

Results and Conclusions: Subclinical hypothyroidism is associated with an increased risk of CHD events and CHD mortality in those with higher TSH levels, particularly in those with a TSH concentration of 10 mIU/L or greater.

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

Nakamura T, Ichimaru S, Ishida N, Soda M (EN), Akahoshi

M (CN), Cullings HM (S), Nakashima E (S), Misumi M (S)

Objectives: To develop and apply the theory of transportability of a regression model; to estimate the risk of metabolic syndrome (MS) related death associated with waist circumference (WC) based on the transportability arguments; and to develop a method for correcting for the measurement error involved in the transported estimates.

Background and Significance: The validity of the diagnostic criteria for MS has been questioned since the syndrome was introduced. To verify the criteria validity, a desirable strategy would be a cohort study with baseline and subsequent measurements. However, WC, which is necessary for the diagnosis of MS, was introduced to health examinations only recently. We considered that it would be possible to estimate the risk associated with WC if an unbiased estimate of WC for each subject 10 years earlier could be obtained.

Study Methods: We first defined statistical transportability, proposed a sufficient mathematical condition, and confirmed the feasibility of the condition with an AHS sample. Then, we obtained a transported estimate of WC for each examinee in the sample 10 years earlier, and applied the Cox regression model with the transported estimate as a covariate. Finally, we developed a method for correcting for the measurement error.

Study Progress: To examine whether WC really decreases as MS-related diseases progress in semi-MS subjects, the transportability was checked for subjects who were in good shape 15 years earlier.

Results and Conclusions: The results of risk estimation for death indicate that, for those of semi-MS (those with at least two indications of: dyslipidemia, hypertension, and impaired glucose tolerance), the larger the WC, the smaller the risk of MS-related death, contrary to expectations. Similar findings were obtained using the full set of AHS study subjects. The results apparently contradict the background assumption in the definition of MS that the larger the WC, the higher the risk of death. Possible causes of the contradictory finding were either that the background assumption of MS was not correct, the data used in the analysis were biased, or the model may require further examination and validation.

Special Clinical Studies Publications

RERF Reports (RR)

◆ Cologne JB, Hsu WL, Abbott RD, Ohishi W, Grant EJ, Fujiwara S, Cullings HM: Proportional hazards regression in epidemiologic follow-up studies: An intuitive consideration of primary time scale. *Epidemiology* 2012 (July); 23(4):565–73. (RR 12-11) (related to *Adult Health Study* and *Tumor and Tissue Registries*) (refer to abstract in *Adult Health Study* Publications)

◆ Neriishi K, Nakashima E, Akahoshi M, Hida A, Grant EJ, Masunari N, Funamoto S, Minamoto A, Fujiwara S, Shore RE: Radiation dose and cataract surgery incidence in atomic bomb survivors, 1986–2005. *Radiology* 2012 (October); 265(1):167–74. (RR 14-11) (refer to abstract in *Adult Health Study* Publications)

◆ Yamada M, Shimizu M, Kasagi F, Sasaki H: Reaction time as a predictor of mortality: The Radiation Effects Research Foundation Adult Health Study. *Psychosom Med*; 2013

(January); 75(2-3):154–60. (RR 25-11) (refer to abstract in *Adult Health Study* Publications)

Other Journal Publications

- ◆ Boffetta P, Hazelton WD, Chen Y, Sinha R, Inoue M, Gao YT, Koh WP, Shu XO, Grant EJ, Tsuji I, Nishino Y, You SL, Yoo KY, Yuan JM, Kim J, Tsugane S, Yang G, Wang R, Xiang YB, Ozasa K, Nagai M, Kakizaki M, Chen CJ, Park SK, Shin A, Ahsan H, Qu CX, Lee JE, Thornquist M, Rolland B, Feng Z, Zheng W, Potter JD: Body mass, tobacco smoking, alcohol drinking and risk of cancer of the small intestine—a pooled analysis of over 500,000 subjects in the Asia Cohort Consortium. *Ann Oncol* 2012 (July); 23(7):1894–8.
- ◆ Imaizumi M: Radiation effects on the thyroid: What has been learned from the atomic bomb radiation and Chernobyl accident. *Proceedings of the FY2011 Radiation Epidemiology Research Meeting*. Tokyo: Radiation Effects Association; 2012, pp 6–8. (Japanese)
- ◆ Kodama K: Epidemiological studies on cardiovascular disease in Hiroshima and Nagasaki, NI-HON-SAN Study, and training of young cardiovascular epidemiologists by conducting teaching seminar. *Nihon Junkankibyō Yobogakkaishi [Jpn J Cardiovasc Dis Prev]* 2013 (January); 48(1):42–50. (Japanese) (related to *Life Span Study* and *Adult Health Study*)
- ◆ Yamada M: Epidemiological study on dementia—Follow up study among atomic bomb survivors. *Ronenki Ninchisho Kenkyukai Shi [Proceedings of the Annual Meeting of the Japanese Research Group on Senile Dementia]* 2012 (August); 19(3):73–5. (Japanese)

Special Clinical Studies Oral Presentations

- ❖ Yanagi M, Abbott RD, Takahashi I, Kawasaki R, Yokoyama T, Fujiwara S, Akahoshi M, Neriishi K, Wang JJ, Kiuchi Y. Association between retinal vessel diameter and smoking: Findings from atomic-bomb survivors in Hiroshima and Nagasaki, Japan. 116th Annual Meeting of the Japanese Ophthalmological Society, 5–8 April 2012, Tokyo
- ❖ Minamoto A, Neriishi K, Nakashima E. UV radiation may explain intercity difference for cataract in atomic-bomb survivors. 27th Asia Pacific Academy of Ophthalmology Congress, 13–16 April 2012, Busan, South Korea
- ❖ Yanagi M, Abbott RD, Takahashi I, Kawasaki R, Yokoyama T, Fujiwara S, Akahoshi M, Neriishi K, Wang JJ, Kiuchi Y. Association between retinal vessel diameter and smoking: Findings from atomic-bomb survivors in Hiroshima and Nagasaki, Japan. 27th Asia Pacific Academy of Ophthalmology Congress, 13–16 April 2012, Busan, South Korea
- ❖ Fujiwara S. Present status and issues of osteoporotic vertebral fracture treatment: Issues from the standpoint of epidemiology of osteoporotic vertebral fracture diagnosis. 41st Annual Meeting of the Japanese Society for Spine Surgery and Related Research, 19–21 April 2012, Kurume
- ❖ Tatsukawa Y, Yamada M, Nakanishi S, Fujiwara S. Review of association of body fat distribution with prevalence of diabetes and metabolic syndrome. 55th Annual Scientific Meeting of the Japan Diabetes Society, 17–19 May 2012, Yokohama (related to *Adult Health Study*)
- ❖ Neriishi K. Cataract in A-bomb survivors. 51st Meeting of the Japanese Society for Cataract Research, 15–17 June 2012, Tokyo

- ❖ Kusumoto S, Kawano H, Koide Y, Ikeda S, Takeno M, Eguchi M, Yonekura T, Akahoshi M, Maemura K. Follow-up of complete right bundle branch block with axis-deviation cases. 112nd Meeting of Kyushu Branch of the Japanese Circulation Society, 30 June 2012, Okinawa
- ❖ Takahashi I, Fujiwara S. Association between height loss and augmentation index. 3rd Conference of Osteobioscience, 14 July 2012, Okayama (related to *Adult Health Study*)
- ❖ Yamada M, Kasagi F, Mimori Y, Miyachi T, Nagano Y, Ohshita T, Sasaki H. Midlife decrease in processing speed linked to dementia occurrence: Radiation Effects Research Foundation Adult Health Study. Alzheimer's Association International Conference on Alzheimer's Disease, 14–19 July 2012, Vancouver, Canada
- ❖ Yanagi M. Association between glaucoma prevalence and retinal vessel diameters. 29th Annual Meeting of the Ocular Circulation Society, 27 July 2012, Akita
- ❖ Akahoshi M, Sera N, Nakashima E. Red blood cell distribution width in hypertensive subjects. 24th Scientific Meeting of the International Society of Hypertension, 30 September–4 October 2012, Sydney, Australia
- ❖ Neriishi K, Abbott RD, Takahashi I, Kawasaki R, Itakura K, Wang JJ, Fujiwara S, Akahoshi M, Yanagi M, Wong TY, Kiuchi Y. Association between retinal vessel caliber and radiation exposure in elderly survivors of the atomic bombings of Hiroshima and Nagasaki. 58th Annual Meeting of the Radiation Research Society, 30 September–3 October 2012, San Juan, Puerto Rico
- ❖ Ohishi W, Fujiwara S, Chayama K. Study of viral hepatitis in a longitudinal cohort of A-bomb survivors. 16th Annual Meeting of the Japan Society of Hepatology, 10–11 October 2012, Kobe (related to *Immunology Studies*)
- ❖ Takahashi I. Assessment of height loss on cardiovascular system. 24th Chugoku District Meeting of the Japan Geriatric Society, 24 November 2012, Hiroshima (related to *Adult Health Study*)
- ❖ Tatsukawa Y, Yamada M, Nakanishi S. Regional body fat distribution and prevalence of diabetes. 9th International Diabetes Federation Western Pacific Region Congress and 4th Scientific Meeting of Asia Association for the Study of Diabetes, 24–29 November 2012, Kyoto (related to *Adult Health Study*)
- ❖ Nakashima E, Neriishi K, Minamoto A. Geographical difference observed in the octant lens opacity data, 2000–2002, from Hiroshima and Nagasaki atomic-bomb survivors. 4th International Conference on Environmental Science and Technology, 30 November–2 December 2012, Bali, Indonesia
- ❖ Fujiwara S, Masunari N, Takahashi I, Ohishi W. Cardiovascular diseases and risk of osteoporotic fracture in a longitudinal cohort study. International Osteoporosis Foundation Regionals—3rd Asia-Pacific Osteoporosis Meeting, 13–16 December 2012, Kuala Lumpur, Malaysia
- ❖ Imaizumi M. Thyroid diseases in A-bomb survivors. Symposium on Thyroid Study, 22 December 2012, Nagasaki
- ❖ Takahashi I, Yanagi M, Misumi M, Kawasaki R, Wang JJ, Wong TY, Neriishi K, Kiuchi Y, Ohishi W. Retinal vessel changes in relation to tobacco cessation—Report from the Adult Health Study, Japan. American Heart Association “Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2013 Scientific Sessions,” 19–22 March 2013, New Orleans, Louisiana, USA

Research Protocols 1-12, 5-89 (Platform Protocol), A2-08**Histopathology Studies****RP 1-12 Development of an archival system for surgical cancer samples from atomic-bomb survivors**

Ozasa K (EH), Sugiyama H (EH), Soda M (EN), Yasui W, Arihiro K, Fujihara M, Arita K, Nishisaka T, Matsuura H, Nakashima M, Shigematsu K, Takahara O, Kusunoki Y (R), Katayama H (IT)

Objectives: To develop an archival system for surgical cancer samples from the atomic-bomb survivors in collaboration with pathologists of the major hospitals in Hiroshima and Nagasaki.

Background and Significance: To clarify the mechanisms of site-specific differences in cancer risks, the shapes of dose-response curves, and the effects of age at the time of bombing and attained age or time since exposure, pathological studies and potentially molecular biological studies of carcinogenesis mechanisms are conducted. These studies should contribute to improved health care for the atomic-bomb survivors and all those suffering from radiation damage in the future.

Study Methods: The major hospitals in Hiroshima and Nagasaki will be collaborators and they shall keep the surgical samples derived from LSS cohort members in accordance with common procedures that are defined in the RP. The guidelines to use the stored samples for research are also defined in the RP.

Study Progress: The RP was approved in January 2012. Preparation of an operations protocol for the procedures between individual hospitals and RERF is being planned.

Results and Conclusions: None yet.

RP 5-89 Pathology studies in Hiroshima and Nagasaki, revised research plan (Formerly RP 3-75)

Ozasa K (EH), Yonehara S, Fujihara M, Soda M (EN), Kodama K (CS)

Objectives: This RP is a revised platform protocol for the conduct of pathology studies at RERF. Pathologists in community and university hospitals are involved in the site-specific cancer incidence studies.

Background and Significance: The RERF autopsy and surgical programs (ABCC TR 4-61 and RERF RP 3-75) were terminated and this RP is the replacement for them.

Study Methods: Intensive efforts are made to seek the cooperation of pathologists at other medical institution in providing pathological materials. We are collecting microscopic slides for those whose autopsies were performed at local hospitals in Hiroshima and Nagasaki since 1989 after terminating the autopsy program at RERF.

Study Progress: A series of site-specific studies has been conducted. We are taking an inventory of formalin-fixed paraffin-embedded tissues that are stored in RERF. Those specimens are from around 7,000 autopsies or surgical operations. We collected 15 slides (5 in Hiroshima and 10 in Nagasaki) this year. To maintain LSS surgical samples, which have been kept in local hospitals, a new RP on "Development of an archival system for surgical cancer

samples from atomic-bomb survivors" (RP 1-12) was established in collaboration with local hospitals and Hiroshima and Nagasaki universities.

Results and Conclusions: Essential methods of collecting and storing pathological materials were established.

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

Nakashima M, Soda M (EN), Furukawa K (S), Sekine I, Yamashita S, Shibata Y, Kodama K (CS)

Objectives: The purpose of this protocol is to identify true multiple primary cancers (MPC) diagnoses by modern histopathological methods as an initial step in evaluating the relationship between MPC and radiation exposure in A-bomb survivors.

Background and Significance: A recent analysis at the Atomic Bomb Disease Institute of the Nagasaki University Graduate School of Biomedical Sciences, using similar histopathological methods, found a strong association between MPC rates and distance from the hypocenter, especially for exposure at young ages. The present study is being conducted within the LSS so that actual dose estimates can be applied to the risk estimation.

Study Methods: Based on immunohistochemistry for cytokeratin-7 (CK7) and CK20 (cytoskeleton proteins), thyroid transcription factor-1 and prostatic specific antigen (tissue-specific markers), lung surfactant PE-10, and ovarian cancer marker CA125, MPC of various sites can be distinguished from metastatic tumors. Pathological review by the principal investigator intends to discriminate metastases from second primary cancers by determining the cell type or tissue of origin.

Study Progress: Among the Nagasaki LSS cohort (N = 38,107), there were a total of 6,305 primary-cancer patients between 1958 and 2003. After reviewing the HE-stained tissue specimens and immunohistochemistry findings among 648 patients who had two or more cancers, 595 cases were identified as MPC, but 41 cases (7%) were not. Pathological specimens of another four cases were inadequate for immunohistochemical staining and specimens of eight cases were not available.

Results and Conclusions: Not yet.

Research Protocols 1-11 and 2-12, 5-10, 5-02 Cell Biology Studies

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

RP 2-12 A study of circulatory diseases using animal models irradiated with lower doses (Addendum to RP 1-11)

Takahashi N, Niwa Y (R), Murakami H (R), Ohishi W (CH), Hsu WL (S), Misumi M (S), Kusunoki Y (R), Inaba T, Nagamachi A, Kokubo T, Oghiso Y, Tanaka S

Objectives: The purpose of this study is to assess the relationship between radiation and circulatory diseases (CD) at doses lower than 4 Gy. Data from pathological analysis and measurement of blood may provide mechanistic information on the relationship between radiation exposure and development of CD.

Background and Significance: Based on the data from the LSS and AHS, we hypothesize that radiation exposure may cause a high risk of CD. To test this hypothesis, we conduct animal studies using the spontaneous hypertensive rat–stroke prone (SHRSP) rats irradiated with 4 Gy or less. This study is expected to deepen our knowledge on the mechanisms of radiation-associated CD.

Study Methods: Male SHRSP rats with age of five weeks are irradiated by gamma ray with 1, 2 or 4 Gy in RP 1-11, and 0.25, 0.5, 0.75 or 1 Gy in a new proposal (RP 2-12) as well as unirradiated control rats. This study is conducted in two ways: 1) The rats are kept until they die to obtain their life span data, and 2) in order to obtain fresh samples for pathological analyses and blood bio-marker measurements, blood samples from the rats are collected immediately after they faint, being followed by sacrifice.

Study Progress: In the study under RP 1-11, we have examined rats irradiated with 1, 2, or 4 Gy, and non-irradiated (0 Gy) rats used as controls. The life span was examined using 20 rats consisting of 5 for each dose group. Eighteen rats were examined for pathological phenotypes to evaluate radiation effects on selected organs. Based on promising results obtained from this study, we have prepared an addendum RP (approved as RP 2-12).

Results and Conclusions: The life span of irradiated rats was significantly shortened compared to control rats. The pathological analyses showed that phenotypic changes observed in the organs (brain, heart, and kidney) of irradiated rats and indicative of hypertension and stroke were more severe than those observed in the same organs of control rats. We have just started studies based on RP 2-12 where we evaluate radiation effects, specifically at lower-dose radiation exposure (≤ 1 Gy). The conclusions will be obtained in early 2015.

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), Ozasa K (EH), Katayama H (IT), Cologne JB (S), Misumi M (S), Izumi S, Oue N, Yasui W, Nakachi K, Kusunoki Y (R)

Objectives: The aim of this study is to clarify molecular oncological characteristics of colon and rectal cancers among A-bomb survivors.

Background and Significance: Radiation exposure is

associated with an increased risk of colon cancer. In colorectal cancer, two major phenotypes, chromosomal instability (CIN) and microsatellite instability (MSI), characterize different carcinogenic pathways. Therefore, we focus on molecular events related to those phenotypes and analyze the molecular oncological characteristics of colorectal cancer among A-bomb survivors. Our hypothesis based on results from a pilot study in 33 cases is that the MSI pathway may preferentially occur in colon cancer among A-bomb survivors, since the five MSI-high (MSI-H) cases showed significantly higher radiation dose than the other 28 cases. This study will help elucidate mechanisms by which there is an increased risk of colon cancer, but not rectal cancer, among A-bomb survivors.

Study Methods: We use archival surgical and autopsied colorectal cancer tissue specimens that are preserved at RERF as well as archival specimens from the Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences, based on protocols B34-03, B35-04. Using DNA extracted from the microdissected cells, we examine MSI and CIN status and their related gene alterations in relation to pathoepidemiological factors including radiation dose.

Study Progress: Of 54 archival colorectal cancer tissue specimens from the LSS subjects, preserved by the Department of Epidemiology at RERF, 49 cases were available for thin slices and completed for H&E-staining and MLH1 protein immunostaining. We started the analyses of MSI and LOH as well as methylation status of *MLH1*. MSI status of 46 exposed cases including 33 cases previously analyzed in pilot study (B38-04) have thus far been determined, and MSI-H phenotype was found in seven exposed cases, of whom five cases did not express MLH1 protein. *MLH1* methylation was observed in six cases with significantly higher dose than *MLH1* unmethylated cases, and four of the six *MLH1* methylated cases showed MSI-H status.

Results and Conclusions: None yet.

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), Taga M (R), Ito R (R), Cologne JB (S), Soda M (EN), Imai K (R), Nakachi K, Kusunoki Y (R)

Objective: To elucidate the mechanisms of adult-onset papillary thyroid carcinogenesis among A-bomb survivors, we will clarify characteristics of gene alterations that occur at an early stage of thyroid carcinogenesis. We will use archival cancer tissue specimens from the LSS subjects.

Background and Significance: Rearrangements of *RET* gene are induced in human thyroid cells by *in vitro* and *in vivo* X-ray irradiation. *RET* rearrangements occur at a low frequency of about 5–10% of sporadic adult-onset papillary thyroid cancer (PTC) cases. On the other hand, we observed that the relative frequency of PTC cases with *RET* rearrangements significantly increased with radiation dose, while the frequency of PTC with point mutations, typically *BRAF*^{V600E}, significantly decreased with dose.

Study Methods: Using DNA and RNA from archival tissue specimens of PTC from the LSS cohort, we examine various gene alterations including *RET/PTC* and *ALK* rearrangements and the *BRAF*^{V600E} point mutation.

Study Progress: Among 25 PTC cases (19 exposed and 6 non-exposed) who carried no alterations in *RET*, *NTRK1*, *BRAF*, and *RAS* genes, a new type of rearrangement, i.e., rearranged anaplastic lymphoma kinase (*ALK*) gene, was found in 10 of 19 exposed PTC cases but none of six non-exposed cases. Rearranged *ALK* gene was not detected in 80 PTC cases with one of *RET*, *NTRK1*, *BRAF* and *RAS* gene alterations, except one PTC case with the *RET* rearrangement which showed extremely low mRNA expression levels of rearranged *ALK* gene. Trabecular/solid-like architectures, which are characterized by solid and/or trabecular appearance, were observed in 6 of 10 PTC cases with *ALK* rearrangements, which was much more frequent than 13 of 95 PTC cases harboring no rearranged *ALK*.

Results and Conclusions: The results suggest that chromosomal rearrangements, such as *RET* and *ALK* rearrangements, play an important role in radiation-associated adult-onset thyroid carcinogenesis. *ALK* rearrangements and other gene alterations are mutually exclusive as an early molecular event of the carcinogenesis.

Cell Biology Studies Publication

RERF Report (RR)

◆ Hamatani K, Mukai M, Takahashi K, Hayashi Y, Nakachi K, Kusunoki Y: Rearranged anaplastic lymphoma kinase (*ALK*) gene in adult-onset papillary thyroid cancer amongst atomic bomb survivors. *Thyroid* 2012 (November); 22(11):1153–9. (RR 15-11) © 2012, Mary Ann Liebert, Inc.

[Abstract] Background: We previously noted that among atomic bomb survivors (ABS), the relative frequency of cases of adult papillary thyroid cancer (PTC) with chromosomal rearrangements (mainly *RET/PTC*) was significantly greater in those with relatively higher radiation exposure than those with lower radiation exposure. In contrast, the frequency of PTC cases with point mutations (mainly *BRAF*^{V600E}) was significantly lower in patients with relatively higher radiation exposure than those with lower radiation exposure. We also found that among ABS, the frequency of PTC cases with no detectable gene alterations in *RET*, neurotrophic tyrosine kinase receptor 1 (*NTRK1*), *BRAF*, or *RAS* was significantly higher in patients with relatively higher radiation exposure than those with lower radiation exposure. However, in ABS with PTC, the relationship between the presence of the anaplastic lymphoma kinase (*ALK*) gene fused with other gene partners and radiation exposure has received little study. In this study, we tested the hypothesis that the relative frequency of rearranged *ALK* in ABS with PTC, and with no detectable gene alterations in *RET*, *NTRK1*, *BRAF*, or *RAS*, would be greater in those having relatively higher radiation exposures. **Methods:** The 105 subjects in the study were drawn from the Life Span Study cohort of ABS of Hiroshima and Nagasaki who were diagnosed with PTC between 1956 and 1993. Seventy-nine were exposed (>0 mGy), and 26 were not exposed to A-bomb radiation. In the 25 ABS with PTC, and with no detectable gene alterations in *RET*, *NTRK1*, *BRAF*, or *RAS*, we

examined archival, formalin-fixed, paraffin-embedded PTC specimens for rearrangement of *ALK* using reverse transcription–polymerase chain reaction and 5′ rapid amplification of cDNA ends (5′ RACE). **Results:** We found rearranged *ALK* in 10 of 19 radiation-exposed PTC cases, but none among six patients with PTC with no radiation exposure. In addition, solid/trabecular-like architecture in PTC was closely associated with *ALK* rearrangements, being observed in 6 of 10 PTC cases with *ALK* rearrangements versus 2 of 15 cases with no *ALK* rearrangements. The six radiation-exposed cases of PTC harboring both *ALK* rearrangements and solid/trabecular-like architecture were associated with higher radiation doses and younger ages at the time of the A-bombing and at diagnosis compared to the other 19 PTC with no detectable gene alterations. **Conclusion:** Our findings suggest that *ALK* rearrangements are involved in the development of radiation-induced adult-onset PTC.

Cell Biology Studies Oral Presentations

❖ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Oue N, Yasui W, Nakachi K, Kusunoki Y. Relationship of MLH1 protein expression with microsatellite instability (MSI) in colorectal cancer among atomic-bomb survivors. 101st Annual Meeting of the Japanese Society of Pathology, 26–28 April 2012, Tokyo

❖ Hamatani K, Mukai M, Takahashi K, Hayashi Y, Nakachi K, Kusunoki Y. Characteristics of gene alterations in papillary thyroid cancer developing in A-bomb survivors. 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima

❖ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Oue N, Yasui W, Nakachi K, Kusunoki Y. Microsatellite instability (MSI) and related gene alterations in colorectal cancer among A-bomb survivors. 21st Annual Meeting of the Japanese Association for Metastasis Research, 12–13 July 2012, Hiroshima

❖ Hamatani K, Mukai M, Takahashi K, Hayashi Y, Nakachi K, Kusunoki Y. Characteristics of adult-onset papillary thyroid cancer with rearranged *ALK* gene in atomic-bomb survivors. 36th Annual Meeting of the European Thyroid Association, 8–12 September 2012, Pisa, Italy

❖ Hamatani K, Takahashi K, Nakachi K, Kusunoki Y. Characteristics of early molecular events in radiation-associated adult-onset papillary thyroid carcinogenesis. 71st Annual Meeting of the Japanese Cancer Association, 19–21 September 2012, Sapporo

❖ Nakachi K. The impact of radiation exposure on the aging human immune system. 25th International Symposium “Radiation and Cancer,” 6–8 December 2012, Tokyo (related to *Immunology Studies*)

Research Protocols 4-11, 1-10, 5-85 and 1-01 Biochemical Genetics Studies

RP 4-11 Genetic study of atomic-bomb radiation by using HD-microarray CGH analysis

Kodaira M (G), Satoh Y (G), Furukawa K (S), Nakamura N, Asakawa J (G)

Objectives: The purpose of the proposed study is to examine if the parental exposure to A-bomb radiation has induced deletion/amplification mutations in the genomes of the F₁ offspring.

Background and Significance: The genetic effects of A-bomb radiation (trans-generational effect) have not been fully elucidated due to low rates of both spontaneous mutation and radiation-induced mutation. We have established the comparative genome hybridization (CGH) approach as a reliable mutation screening method by improving the repeatability, resolution, accuracy, and efficiency. Our improved CGH approach can now detect from small (3–5 kb) to large (~10 Mb) deletions with high accuracy. Since radiation-induced mutations are primarily deletions initiated with DNA double-strand breaks, we propose a CGH study using high-density microarrays.

Study Methods: We will analyze a total of 688 DNA samples from both parents of 184 families (exposure is restricted to either parent) and 320 offspring (160 from paternally and 160 from maternally exposed families).

Study Progress: Establishing a database of the polymorphic copy number variations (CNVs) in this population is indispensable to identify candidate low-frequency mutations. We first examined 26 sets of mother-father-child trio samples, 13 each from Nagasaki and Hiroshima, and made a list of polymorphic CNVs. We expanded the CGH examination to another 70 trio samples.

Results and Conclusions: Not yet obtained.

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), Shimada Y, Cullings HM (S), Nakamura N

Objectives: The purpose of the study is, as an animal model of human male exposure, to estimate the mutation induction rate following 4 Gy of gamma irradiation of mouse spermatogonia, and to molecularly characterize the mutations.

Background and Significance: 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 and whether both are of equal importance) from the offspring of Hiroshima and Nagasaki survivors whose radiation doses are much smaller than those used in animal studies.

Study Methods: We will estimate the mutation induction rate by examining DNA samples of 80 F₁ mice derived from the spermatogonia of male mice irradiated with 4 Gy of rays and 80 F₁ mice in the control group by an HD-array CGH technique.

Study Progress: A total of 21 mutations, 9 in the exposed group (6 deletions in 6 mice, 3 amplifications in 4 mice) and

12 mutations in the control (7 deletions in 7 mice, 5 amplifications in 3 mice, i.e., 1 mouse had 3 amplifications) were detected. We molecularly characterized most of the deletion mutations, including junction sequencing and identification of parental origin by SNP analyses.

Results and Conclusions: The results imply that the frequency of large deletions induced by radiation exposure may be considerably lower than currently estimated.

RP 5-85 Culture of lymphoblastoid 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)

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

Objectives: To archive untreated cells and to establish lymphoblastoid cell lines by transformation of B-cells from members (parents and children) of families as biological resources for current and future genetic studies, and to obtain written informed consents from them.

Background and Significance: Blood cells have been obtained from 908 parent-child “trios” (including 1,500 children) and lymphoblastoid cell lines have been established. Moreover, as the new F₁ clinical follow-up study has started from November 2010, we have started to re-collect the blood samples from the participants in order to increase the numbers of untreated cells, which will be useful for analyses by newly developed technologies in the future.

Study Methods: An aliquot of lymphocytes separated from the donated blood was transformed by Epstein-Barr virus to establish cell lines while the remaining part of lymphocytes and poly-nuclear cells were cryo-preserved without culturing. In the new F₁ clinical study, additional uncultured lymphocytes and poly-nuclear cells will be cryo-preserved.

Study Progress: We collected new blood samples from 150 individuals this year.

Results and Conclusions: In the course of this project, we have established cell lines from 4,374 individuals with informed consents. The total number of children who gave us informed consents and whose cell lines were established is 1,966 as summarized on the following page (Table) in relation to parental dose. And we have re-collected 299 specimens in the new F₁ clinical study. The numbers of re-collected blood samples are summarized inside the parentheses of the Table.

Biochemical Genetics Studies Publication

RERF Report (RR)

◆ Asakawa J, Kodaira M, Cullings HM, Katayama H, Nakamura N: The genetic risk in mice from radiation: An estimate of the mutation induction rate per genome. *Radiat Res* 2013 (March); 179(3):293–303. (RR 9-12) © 2013 by Radiation Research Society

[Abstract] Restriction Landmark Genome Scanning (RLGS) is a method that uses end-labeled ³²P *NotI* sites that are mostly associated with coding genes to visualize thousands of DNA fragments as spots in two-dimensional

Table. The total number of children with informed consents and cell lines

Father's dose (Gy)	Mother's dose (Gy)							Total
	>2.00 ^a	1.50–1.99	1.00–1.49	0.50–0.99	0.01–0.49	0&Null	Unknown	
>2.00	2(1 ^b)	3		1	4	66(12)	1	77(13)
1.50–1.99					3	44(12)	2	49(12)
1.00–1.49		1	7	5	17	96(19)	3	129(23)
0.50–0.99		2(1)	1	13(3)	8	132(24)	4	160(28)
0.01–0.49	4	2	2	11	41(7)	113(27)	5(1)	178(35)
0&Null	27(5)	44(5)	117(21)	292(46)	137(28)	653(81)	51	1,321(186)
Unknown	2			7(2)	4	39		52(2)
Total	35(6)	52(6)	127(21)	329(51)	214(39)	1,143(175)	66(1)	1,966(299)

^a The doses shown in the table are rounded at the second decimal place.

^b The numbers in parentheses are the number of children whose blood samples have been re-collected.

autoradiograms. This approach allows direct detection of autosomal deletions as spots with half normal intensity. The method was applied to mouse offspring derived from spermatogonia exposed to 4 Gy of X rays. A genome-wide assessment of the mutation induction rate was estimated from the detected deletions. Examinations were made of 1,007 progeny (502 derived from control males and 505 from irradiated males) and 1,190 paternal and 1,240 maternal spots for each mouse. The results showed one deletion mutation in the unirradiated paternal genomes of 502 offspring (0.2%) and five deletions in the irradiated paternal genomes of 505 offspring (1%). The difference was marginally significant, with the deletion sizes ranged from 2–13 Mb. If the frequencies are taken at face value, the net increase was 0.8% after an exposure of 4 Gy, or 0.2% per Gy per individual if a linear dose response is assumed. Since the present RLGs analysis examined 1,190 *NotI* sites, while the mouse genome contains ~25,000 genes, the genomic probability of any gene undergoing a deletion mutation would be $25 \times 0.2\%$, or 5% per Gy. Furthermore, since the present RLGs screened about 0.2% of the total genome, the probability of detecting a deletion anywhere in the total genome would be estimated to be 500 times 0.2% or 100% (i.e., 1 deletion per Gy). These results are discussed with reference to copy number variation in the human genome.

Biochemical Genetics Studies Oral Presentations

- ❖ Asakawa J. Genetic study of radiation on the offspring of the A-bomb survivors (genetic study at DNA level). The 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima
- ❖ Miura A, Tsuji T, Imanaka M, Nakamoto Y, Kodaira M, Nishimura M, Shimada Y, Asakawa J. Spontaneous and radiation-associated germ cell mutations in mice detected by high-density microarray CGH. 37th Annual Meeting of the Chugoku Area Radiation Research Society, 27 July 2012, Hiroshima
- ❖ Asakawa J, Nakamura N. Look at the data obtained by the genetic study of radiation on mice. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai
- ❖ Imanaka M, Nakamoto Y, Miura A, Tsuji T, Kodaira M, Furukawa K, Cullings HM, Nishimura M, Shimada Y, Asakawa J. Detection of spontaneous and X-ray induced

germ cell mutations in mice by high-density microarray CGH. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai

- ❖ Kodaira M, Miura A, Imanaka M, Tsuji T, Nakamoto Y, Nishimura M, Shimada Y, Asakawa J. Molecular characterization of spontaneous and radiation-associated deletion mutations in mouse germ cells. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai
- ❖ Takahashi N, Niwa Y, Murakami H, Ohishi W, Hsu WL, Nagamachi A, Inaba T, Oghiso Y, Tanaka S, Kokubo T, Kusunoki Y. Study of radiation induced circulatory disease using animal models. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai
- ❖ Asakawa J, Miura A, Nakamoto Y, Tsuji T, Imanaka M, Kodaira M, Nishimura M, Shimada Y, Nakamura N. Estimation of transgenerational effects of radiation in male germ cells of mice using high-density microarray CGH platform. 39th Annual Meeting of the European Radiation Research Society, 15–19 October 2012, Vietrisul Mare, Italy
- ❖ Asakawa J. Transgenerational effects of radiation. JRRS Workshop “Biological Effects of Exposure to Low-dose Radiation,” 30–31 October 2012, Fukushima
- ❖ Niwa Y, Takahashi N. Whether lower dose radiation can induce circulatory disease?—Validation using animal models. FY2012 Research Meeting of research to affect the health effect of the radiation, 6 February 2013, Tokyo

Research Protocols 6-11, 6-09, 1-08, 6-00, 8-93, A4-09, A2-09

Cytogenetics Studies

RP 6-11 A study of chromosome aberration frequency in thyroid cells following fetal exposure to ionizing radiation in mice

Hamasaki K (G), Noda A (G), Nakamura N, Hsu WL (S), Kodama Y (G)

Objectives: Aims of this study are to examine whether chromosome aberrations induced by *in utero* exposure to radiation persist in mouse thyroid cells and to better understand the tissue-specific characteristics of radiation-induced chromosome aberrations following fetal irradiation.

Background and Significance: Studying the biological effects of radiation to fetuses is essential for understanding the cancer risk to the fetus. We have previously reported that chromosome aberration frequency following fetal exposure to radiation may differ by tissues; namely, it is low in lymphoid cells while remaining high in mammary epithelial cells in rats. To understand the mechanisms of the tissue dependency, we propose to examine thyroid cells of mice following fetal irradiation to see whether or not the radiation effect remains recorded. The results obtained in this study may help to explain the cancer risks of A-bomb survivors who were exposed *in utero*.

Study Methods: To test the hypothesis that chromosome aberration frequency following fetal irradiation may differ by tissues, we will examine translocation frequency in mouse thyroid cells in three groups of mice; that is, 1) mice irradiated as fetuses, 2) mothers irradiated while pregnant, and 3) non irradiated mice. Using chromosome slides prepared following primary culture of thyroid epithelial cells, translocation frequencies will be determined and evaluated by a FISH (fluorescence *in situ* hybridization) method that labels chromosome 1 (green) and 3 (red). More than 500 metaphase cells will be needed for analysis in each group.

Study Progress: We successfully determined suitable conditions for both primary culture and FISH staining of cultured mouse thyroid cells. Preliminary data indicated that there were 12 translocations in 530 metaphase cells in the fetus and 12 translocations in 342 cells in their irradiated mother, while no aberrations were observed in control mice (0/519).

Results and Conclusions: Preliminary data suggested that chromosome damage induced following fetal exposure to radiation in mouse thyroid cells persists as it does in mammary epithelial cells. Further analyses will be done in 2013.

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, 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)

Objectives: To evaluate the relative risk of developing skin cancers among carriers of an inactive allele of the XPA

genes.

Background and Significance: The frequency of patients with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is very rare, but carriers (heterozygotes) are not rare. However, there are few data regarding cancer risk in heterozygote carriers, as they are generally difficult to identify. This study will focus on a founder mutation allele of the XPA gene, which is an inactive allele known to cause severe disease phenotypes under homozygous conditions and the mutation heterozygotes are found in about 1% of the population, which is unique to the Japanese. Those conditions provide a unique advantage in effective screening of such carriers.

Study Methods: We will screen about 1,000 non-melanoma skin cancer specimens and an additional 500 chromosome slides as the control to estimate the frequency of the XPA heterozygotes using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Study Progress: We screened DNA from an additional 180 chromosome slides and 60 non-melanoma skin cancers. We found no additional XPA heterozygotes among them.

Results and Conclusions: Thus far, we found five XPA heterozygotes among 682 chromosome slides, and seven XPA heterozygotes among 476 non-melanoma skin cancers.

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

Objectives: To make novel animal model systems which will allow us to estimate the genetic risk (mutagenic risk) of radiation at low doses. Risks of somatic mutations and genomic instabilities in various tissues will also be validated in irradiated individuals as well as F₁ populations born to irradiated parents.

Background and Significance: Since the genetic risk of radiation is difficult to assess at low doses, we plan to generate new mouse *in vivo* systems to detect and measure radiation-induced mutant frequencies occurring in germ cells, as well as somatic cells, at low doses. In these genetically engineered mice, mutant cells that are naturally-occurring, or radiation-induced, become fluorescent in living tissues. We can analyze the mutant cell frequencies in various tissues at the whole body level. Genetic risk of radiation is derived from mutations in spermatogonia stem cells. Our systems will allow us to count spermatogonia cell mutants by distinguishing them from auxiliary cells that constitute stem cell niche structure, or large numbers of cells could be analyzed quickly by flow cytometry. That is a breakthrough in classical genetics, which had to analyze millions of F₁ mice. With regards to somatic tissue, mutagenic risk assessment for target organs of radiation-carcinogenesis will be validated *in situ*, without destroying tissue's three-dimensional structure.

Study Methods: By applying mouse embryonic stem (ES) cell knock-in techniques, recombinant mice will be generated. We plan to create two kinds of mouse strains whose radiation-induced mutant cells become fluorescent so that they may be easily detected under microscopic observation and flow cytometry. One is a gene reversion system; rever-

sion from a tandem duplication of specific gene locus makes the cells produce green fluorescent protein (GFP). We generated mice carrying a partial duplication of the *HPRT* gene in conjunction with the *GFP* gene at the 3' end of the duplicate (*HPRT^{dup}GFP* mice). In this case, reversion from the duplicate produces *HPRT-GFP* fusion proteins, thereby making the mutant cells fluorescent. The other is a gene forward mutation system; activating mutations of oncogenes, or inactivating mutations of tumor suppressor gene, makes the cells GFP positive. In these cases, radiation-induced mutation will directly lead to the development of tumors. We plan to make such systems by using the *ras* or *p53* gene.

Study Progress: We have successfully made *HPRT^{dup}GFP* mice. In these mice, spontaneously arising and radiation-induced mutant cells (revertants) in the body could be detected clearly by observation of dissected tissues with a fluorescent microscope. We confirmed it with the pancreas, liver, small intestine, spleen, and thyroid gland. Therefore now almost all organs or tissues of mice can be used for risk assessment following radiation exposure. As a first step toward the goal, 3-Gy-irradiated mice at three months are examined, and we found 2–3 fold induction of somatic mutations in the pancreas and liver. Mutations at intestinal stem cells (crypt stem cells) produced the images of linear stripes of mutant cells from the crypt bottom to the top of the intestinal villus. We successfully detected an increased frequency of mutant cells in the irradiated small intestine. Mutation induction in spleen lymphocytes was also detected using a flow-cytometric method. Mutations of spermatogonia stem cells were somewhat fuzzy under microscopic observations. Therefore we switched to analysis with flow cytometry by using spermatogonia stem cell markers, CD9 and CD322, and detected an increased frequency of mutant stem cells.

Results and Conclusions: Although it took several years to succeed at mouse knock-in experiments in our institute, now the first generation of knock-ins that can be used for the study of radiation effects are firmly established. We held an international workshop on “radiation-induced stem cell mutations” early this year in Hiroshima. In September, we presented our results at American Environmental Mutagen Society meeting and discussed with Dr. Engelward of MIT, who was the first to establish such *in vivo* mutant detection systems in mice (*Proc Natl Acad Sci USA* 2008; 105:10314–9).

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

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

Objectives: 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.

Background and Significance: Breast cancer is one of the most radiogenic tumors among A-bomb survivors. Further, the risk is particularly high among those who were exposed at ages below 20 and developed breast cancer

before age 35 (early onset). We hypothesized that the high risk is due to the heterozygous inheritance of mutated breast cancer susceptibility genes and that radiation exposure had caused loss of the function of the remaining wild-type allele.

Study Methods: We will examine the plausibility of specific single nucleotide polymorphisms (SNPs) that have been suggested as contributing to early-onset breast cancers.

Study Progress: We finished the examination of the TP53 codon72 polymorphism among 589 breast or ovary cancer cases

Results and Conclusions: None yet.

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), Kusunoki Y (R), Shimizu Y (EH), Cullings HM (S), Misumi M (S), Nakamura N

Objectives: The purpose of this study is to examine quantitatively radiation-induced chromosome aberrations (mainly translocations) in blood lymphocytes of atomic-bomb (A-bomb) survivors in the Adult Health Study (AHS) cohort using the fluorescence *in situ* hybridization (FISH) technique.

Background and Significance: A cytogenetic investigation following ionizing radiation exposure is regarded as the most reliable tool for biological dosimetry. Blood lymphocytes can be considered as a natural, *in situ* dosimeter for the exposed individual. A further advantage is that the chromosome test is free from any recall bias, while physical dose reconstruction requires precise information on location and shielding conditions for individual dose estimation that are critical but are often unavailable or subject to recall biases.

Study Methods: We have employed 2-color-FISH for detection of translocations involving chromosomes 1, 2, and 4. As a routine procedure, we score 500 FISH-stained metaphases per sample. All blood samples are coded so that the chromosome tests are being performed without any knowledge of individual physical doses.

Study Progress: In FY2012, blood samples were examined from 63 survivors (41 from Hiroshima and 22 from Nagasaki). To date, 1,142 survivors in Hiroshima and 681 survivors in Nagasaki have been examined with FISH.

Results and Conclusions: The dose response from current FISH data show a wide scattering of individual translocation frequencies as we observed in the previous solid Giemsa staining study. However, the two sets of data (solid Giemsa and FISH) for the same individuals agreed well with each other.

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

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

Objectives: We hypothesized that radiation-induced unreparable DNA double strand breaks (DSB) are permanently retained in non-apoptotic, non-dividing quiescent cells. We will find sensitive methods to detect such unreparable DSB *in vitro* and *in vivo*, and apply them to archival tissues of A-bomb survivors so that retrospective dose estimation can be made.

Background and Significance: Following irradiation of cells *in vitro*, unrepairable DSB form large foci consisting of phosphorylated ATM/ γ H2AX/53BP1/ubiquitinated proteins in the nuclei, months after the exposure. Possibly they remain years. If we are able to detect such foci in the tissues exposed to radiation long ago, it will help us to estimate radiation doses, as well as to elucidate causative mechanisms of late effects of radiation in irradiated tissues.

Study Methods: (1) We will biochemically isolate and dissect the protein components of the unrepairable DSB-foci, then (2) determine specific proteins that distinguish the unrepairable DSB-foci so as to make specific antibodies against the proteins.

Study Progress: A long time after exposure to ionizing radiation (even after one year) we are able to successfully detect unrepairable DSB-foci in a dose dependent manner in normal human fibroblasts which were kept under confluent conditions. We also detected such damage in irradiated mouse tissues. We could estimate accumulated dose by counting the unrepairable DSBs. The cells bearing the damage underwent premature senescence, indicating that cell aging was accelerated. Senescent cells, both naturally occurring and radiation-induced, show deformed shape in the nuclear envelope. Therefore we hypothesized that the formation of unrepairable DSBs caused dysfunctional nuclear membrane architecture. Indeed, mitigation of deformation of the nuclear membrane by chemicals or introduction of the telomerase gene reduced the radiation-induced unrepairable DSBs.

Results and Conclusions: Our results indicate that the nuclear membrane is involved in DSB repair, especially for such damage that is difficult to be subjected to normal repair. Since the cells carrying the unrepairable DSBs undergo premature senescence, tissues or organs consisting of non-apoptotic cells (apoptosis resistant cells such as connective tissue cells) could retain the damage, which we speculate is one of the causative mechanisms of tissue accelerated aging as a late effect of radiation.

RP-A2-09 Comprehensive analysis of radiation-induced genetic damage 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)

Objectives: Comprehensive analysis of damaged genome regions following radiation exposure in human peripheral blood T-cell populations.

Background and Significance: Ionizing radiation induces various types of structural alterations in the genome, and some genetic alterations may lead to cell transformation. However, little is known about how large chromosomal regions are altered and what types of genetic alterations preferentially remain in a normal cell following ionizing irradiation with a given dose. This approach may provide a novel way to seek possible mechanisms of somatic gene mutations leading to radiation-related cancers.

Study Methods: To determine structural alterations in the genome following radiation exposure, we will evaluate damaged genome regions in human peripheral blood T-cell populations that will be clonally propagated after *in vitro*

X-irradiation. Damaged genome regions will be identified by a comparative genomic hybridization (CGH) assay using a 244K human genome array that allows comprehensive analysis over the entire genome. G-banding and multi-color chromosome analyses will also be conducted for T-cell clones identified with gene deletions to confirm damaged genome regions at the chromosomal level.

Study Progress: All assays have been completed.

Results and Conclusions: Among 33 T-cell clones from peripheral blood mononuclear cells irradiated with 1 Gy of X rays *in vitro*, 11 (33%) showed structural changes including deletions (14 kb–130 Mb) or amplification (28 kb) at least in one chromosome, and one clone showed trisomy. Molecular and cytogenetic analyses demonstrated that the majority of deletions were simple interstitial ones, and that one clone has a large terminal deletion (130 Mb) accompanying an unbalanced translocation. Fourteen unirradiated T-cell clones were similarly analyzed: One showed trisomy and the other 13 showed no structural changes. This result indicates that genome damages induced by X ray in normal human blood cells are usually repaired by non-homologous end-joining, resulting in simple interstitial deletions.

Cytogenetics Studies Publications

RERF Reports (RR)

◆ Nakano M, Kodama Y, Ohtaki K, Nakamura N: Translocations in spleen cells from adult mice irradiated as fetuses are infrequent, but often clonal in nature. *Radiat Res* 2012 (December); 178(6):600–3. (RR 7-12) © 2012 by Radiation Research Society

[Abstract] We previously reported that mouse fetuses or neonates exposed to 2 Gy of X rays showed an unexpectedly low incidence of chromosome damage in lymphocytes, bone marrow, and spleen cells when the mice were subsequently examined at 20 weeks of age. However, cells bearing translocations were occasionally observed that, on the basis of 2-color whole chromosome painting, appeared to be clonal descendants. Unfortunately, this approach typically did not permit unequivocal confirmation of their clonality. To overcome this problem, multi-color FISH (mFISH) was employed, which assigns all 21 individual chromosome types of the mouse a unique color. After mFISH analyses of the same cell samples studied previously, it was confirmed that spleen cells of 20-week-old mice irradiated either as 15.5-day fetuses or as 3- to 4-day-old neonates showed translocation frequencies close to zero. Translocations previously suspected as being clonal in nature were confirmed as such by mFISH, which also revealed the presence of an additional clone not previously detected or suspected. Since no evidence of clonality was observed in the irradiated mother, we concluded that in both fetuses and neonates, there exists a small fraction of stem cells that are distinct from the bulk of the stem cell compartment in terms of their ability to acquire and transmit radiation-induced chromosome damage through clonal expansion.

◆ Noda A, Hirai Y, Hamasaki K, Mitani H, Nakamura N, Kodama Y: Unrepairable DNA double-strand breaks that are generated by ionising radiation determine the fate of normal human cells. *J Cell Sci* 2012 (November); 125(22):5280–7. (RR 10-11) © 2012 Published by the Company of Biologists

Ltd. (This abstract was reprinted by permission of the Company of Biologists Ltd.)

[Abstract] After an exposure to ionising radiation, cells can quickly repair damage to their genomes; however, a few unreparable DNA doublestrand breaks (DSBs) emerge in the nucleus in a prolonged culture and perpetuate as long as the culture continues. These DSBs may be retained forever in cells such as non-dividing ageing tissues, which are resistant to apoptosis. We show that such unreparable DSBs, which had been advocated by the classical target theory as the 'radiation hit,' could account for permanent growth arrest and premature senescence. The unreparable DSBs build up with repeated irradiation, which accounts for an accumulated dose. Because these DSBs tend to be paired, we propose that the untethered and 'torn-off' molecular structures at the broken ends of the DNA result in an alteration of chromatin structure, which protects the ends of the DNA from genomic catastrophe. Such biochemical responses are important for cell survival but may cause gradual tissue malfunction, which could lead to the late effects of radiation exposure. Thus, understanding the biology of unreparable damage will provide new insights into the long-term effects of radiation.

Other Journal Publications

- ◆ Hamasaki K: Radiation effects on the human body. *Sangyo Hoken 21 [Occup Health 21]* 2013 (January); 18(3):5–7. (Japanese)
- ◆ Kodama Y, Noda A, Booth C, Breault D, Suda T, Hendry J, Shinohara T, Rube C, Nishimura EK, Mitani H, Nakamura N, Niwa O: International workshop: Radiation effects on mutation in somatic and germline stem cells. *Int J Radiat Biol* 2012 (June); 88(6):501–6.
- ◆ Nakamura N, Nakano M, Hamasaki K, Ohtaki K, Sakata R, Sugiyama H, Noda A, Kodama Y: Is it true that radiation-associated risks are higher following irradiation of fetuses as compared with children? *Hoshasen Seibutsu Kenkyu [Radiat Biol Res Commun]* 2012 (September); 47(3):272–86. (Japanese)
- ◆ Shi L, Fujioka K, Sun J, Kinomura A, Inaba T, Ikura T, Ohtaki M, Yoshida M, Kodama Y, Livingston GK, Kamiya K, Tashiro S: A modified system for analyzing ionizing radiation-induced chromosome abnormalities. *Radiat Res* 2012 (May); 177(5):533–8.

Cytogenetics Studies Oral Presentations

- ❖ Hamasaki K, Noda A, Nakamura N, Kodama Y. A study for chromosome aberration frequency following fetal irradiation in thyroid epithelial cells of mice. 37th Annual Meeting of the Chugoku Area Radiation Research Society, 27 July 2012, Hiroshima
- ❖ Noda A, Mishima S, Hirai Y, Hamasaki K, Mitani H, Michaelis S, Kiyono T, Nakamura N, Kodama Y. Functional associations between radiation-induced unreparable DSBs and senescence accelerating gene (Progerin). 37th Annual Meeting of the Chugoku Area Radiation Research Society, 27 July 2012, Hiroshima
- ❖ Hamasaki K, Noda A, Nakamura N, Kodama Y. Chromosome aberration frequency following fetal irradiation depends on the tissue. 55th Annual Meeting of the Japan

- Radiation Research Society, 6–8 September 2012, Sendai
- ❖ Mishima S, Noda A, Hirai Y, Hamasaki K, Nakamura N, Kodama Y. Progerin, a protein that is responsible for human progeroid syndrome, cause to increases radiation-induced unreparable DSBs. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai
- ❖ Noda A, Suemori H, Hirai Y, Kodama Y, Nakamura N. HPRTdup-GFP mice: a new knock-in mouse system for the in vivo visualization of somatic and germ cell mutants. 43rd Annual Meeting of Environmental Mutagen Society, 8–12 September 2012, Bellevue, Washington, USA
- ❖ Ukai A, Hamasaki K, Kodama Y, Noda A, Kusunoki Y, Honma M. Genome mapping of damaged chromosome regions in normal human cells induced by ionizing irradiation using CGH-microarray analysis. 41st Annual Meeting of the Japanese Environmental Mutagen Society, 29–30 November 2012, Shizuoka
- ❖ Noda A, Hirai Y, Hamasaki K, Nakamura N, Kodama Y. Implication of radiation-induced unreparable DSBs in late effects of radiation and cell aging. 3rd RIRBM (Hiroshima University Research Institute for Radiation Biology and Medicine) International Symposium "Biological Effects of Low Dose Radiation," 12–13 February 2013, Hiroshima

**Research Protocols 3-02, 4-75 (Platform Protocol)
F₁ 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

Grant EJ (EH), Furukawa K (S), Sakata R (EH), Ozasa K (EH), Kodama K (CS), Watanabe T (EH), Fujiwara S, Cologne JB (S)

Objectives: To ascertain baseline epidemiological data on the F₁ subjects in order to investigate possible confounding or effect-modifying risk factors in evaluating possible relationships of adult-onset diseases and parental exposure to ionizing radiation from atomic bomb radiation. Secondly, to identify and enlist F₁ cohort members willing to participate in clinical health examinations.

Background and Significance: Studies of genetic effects have been a primary focus of the ABCC and RERF research program since its inception more than 60 years ago, including surveillance of the mortality experience and cancer incidence among a large fixed cohort of children of atomic bomb survivors (F₁). These early studies have not, as yet, provided evidence of dose-related genetic damage in the children of exposed parents, but as of the last published report the children were still relatively young (mean ages <50), so most of their disease experience is yet to come. In order to more accurately assess radiation risk for mortality (cancer and noncancer) and cancer incidence, it is desirable to obtain lifestyle and other potential confounder information for the F₁ cohort.

Study Methods: A self-administered mail survey was conducted among a stratified random subset of the F₁ mortality cohort.

Study Progress: The mail survey was sent to 24,673 people (13,389 males and 11,284 females) between 2000 and 2006. At the end of the survey, 16,756 (68%) subjects responded. We made a final-report brochure that summarized the F₁ clinical study and sent it to the survey respondents in December 2008 as a thank-you for their participation.

Results and Conclusions: A total of 14,145 (57%) subjects among the mail survey subjects agreed to participate in the RERF Clinical Studies Department's health examination and 11,951 (71% of questionnaire respondents) actually participated in the F₁ clinical examination. Lifestyle information from the questionnaire (smoking and drinking habits) was analyzed in the F₁ clinical study and a paper including the mail survey data from the clinic subset was published in 2008 (Fujiwara S, Suyama A, et al., *Radiat Res* 170(4):451–7).

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

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

Objectives: To determine whether parental radiation exposure affects mortality and/or cancer incidence rates in their

offspring.

Background and Significance: Animal studies have reported effects of radiation on mutation rates in the F₁ generation in accordance with the genetic hypothesis. This population provides one of the very few opportunities in the world to study the heritable genetic risks in humans from exposure to ionizing radiation. As population exposures to medical radiation procedures increase before and during childbearing years, the public health implications of the results from this study continue to be timely and important.

Study Methods: The F₁ mortality cohort target sample consisted of 76,814 subjects. They were selected from among 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 to acute radiation. A-bomb exposure doses to both parents could be estimated for about 41,000 offspring with follow-up.

Study Progress: The most recent published scientific manuscripts for this study included mortality through 1999 and cancer incidence through 1997. Analyses of updated cancer and noncancer mortality (through 2008) are underway.

Results and Conclusions: To date, we have found no significantly increased rates of noncancer or cancer mortality, or cancer incidence associated with paternal or maternal dose.

F₁ Studies Oral Presentations

- ❖ Grant EJ. Studies of radiation risks in the children of the atomic-bomb survivors. 37th Annual Meeting of the Chugoku Area Radiation Research Society, 27 July 2012, Hiroshima
- ❖ Grant EJ, Furukawa K, Sakata R, Suyama A, Ozasa K. Mortality experience of the children of atomic-bomb survivors: An update after 57 years of follow-up. 58th Annual Meeting of the Radiation Research Society, 30 September–3 October 2012, San Juan, Puerto Rico

Research Protocols 7-11, 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 and 5-11, 9-88, 29-60, A5-12, A3-11, A5-10, A3-10, A12-08, A5-08

Special Cancer Studies

RP 7-11 Preservation of fresh thyroid samples obtained from Adult Health Study participants (Addendum to RP 2-86)

Imaizumi M (CN), Ohishi W (CH), Sera N (CN), Hida A (CN), Yamada M (CH), Hamatani K (R), Ozasa K (EH), Akahoshi M (CN)

Objectives: To preserve fresh thyroid samples removed from thyroid tumor cases detected among AHS subjects for future genetic and mechanistic studies on thyroid radiation carcinogenesis.

Background and Significance: Thyroid cancer shows an increased incidence with radiation dose among A-bomb survivors. However, the mechanisms of radiation induction of thyroid cancer are not fully understood. Collection of as many thyroid cancer samples as possible, would contribute to the mechanistic elucidation of thyroid cancer development and radiation carcinogenesis. Furthermore, since significant dose response is observed even for benign thyroid tumors, it is also important to compare cancer and benign tumors at the molecular level for elucidation of carcinogenic mechanisms. In accordance with an earlier research protocol RP 2-86, RERF has worked on collection and cryopreservation of fresh thyroid cancer tissues from A-bomb survivors and nonexposed controls. Thyroid ultrasound examination has been performed in AHS health examinations since 2000, thereby leading to the detection of a large number of thyroid nodules. On account of the rapid decrease in the number of A-bomb survivors, it is important to collect systematically as many thyroid samples as possible, regardless of benign or malignant thyroid tumors, from AHS subjects. The present RP, an addendum to the aforementioned RP 2-86, aims at the systematic collection of both fresh benign and malignant thyroid tumor samples removed from AHS subjects.

Study Methods: When we are notified that thyroid surgery is to be performed in an AHS subject, we obtain advance consent from the donor subject and visit the hospital to obtain the fresh sample. One to two pieces of the sample will be rapidly frozen in liquid nitrogen.

Study Progress: We have started sample collection, and samples from one thyroid cancer case have been obtained this year.

Results and Conclusions: None. This RP is only for collection of frozen fresh samples from thyroid tumor cases detected among AHS subjects in preparation for future molecular research. A research protocol using the samples will be prepared separately in the future.

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

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

Objectives: To evaluate the combined effects of A-bomb radiation and subsequent therapeutic radiation.

Background and Significance: Very limited information is available from epidemiological studies on the effects of exposure to multiple radiation sources. The RP focuses on secondary cancer risk of a subset from the LSS who had undergone radiotherapy subsequent to A-bomb radiation.

Study Methods: The subjects include 1,501 A-bomb survivors who were confirmed to have undergone radiotherapy and whose organ or tissue doses from that radiotherapy will be approximated. Secondary cancer risks from radiotherapy doses will be calculated adjusting for A-bomb radiation dose and also modifying A-bomb radiation effects.

Study Progress: Analysis is ongoing at the National Institute of Radiological Sciences (NIRS).

Results and Conclusions: None yet.

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), Cologne JB (S), Fujiwara S, Ueda K (CH), Akahoshi M (CN), Niwa Y (R), Ozasa K (EH), Tsuge M, Chayama K

Objectives: 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.

Background and Significance: This research protocol is an addendum to RP 1-04. 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.

Study Methods: We will measure blood cytokine levels related to chronic inflammation and/or insulin resistance that are considered important factors contributing to acceleration of the development of HCC using stored sera before HCC diagnosis among subjects of this nested case-control study (RP 1-04), as well as examine their contribution to HCC risk.

Study Progress: We have estimated the relationship between inflammatory marker levels and HCC risk based on the tertile distribution of each marker in the controls, after adjusting for confounding factors such as HBV and HCV infection, lifestyle-related factors, and radiation dose. A paper on serum inflammatory markers associated with HCC risk has been submitted to an international journal.

Results and Conclusions: Higher serum levels of IL-6 were associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-related factors, and radiation dose. The association is especially pronounced among subjects with obesity.

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), Sekine I, Mabuchi K, Preston DL, Sadakane A (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and breast cancer incidence based on histologically reviewed diagnoses according to current WHO classification criteria, and to clarify the histoimmunological characteristics of radiation-induced breast cancer. This study updates the past study by adding cases from 1991–2005 and will provide more precise estimates of radiation effects.

Background and Significance: Breast cancer shows one of the highest associations with radiation, but no differences in the histological distributions were observed in the past between exposed and control cases. Histological reviews according to a new classification system along with histoimmunological subtyping will permit a re-evaluation.

Study Methods: Potential breast cancer cases during 1950–2005 collected from cancer registries and other available sources will be histologically reviewed by pathologists. Intrinsic subtypes of breast cancer according to estrogen and progesterone receptors (ER/PR) and Her2 will be investigated (RP 6-10).

Study Progress: Histopathological review has been completed by two pathologists for approximately 90% of 1,732 probable cases. The histoimmunological staining to determine intrinsic subtypes has been completed for around 500 cases among the histologically confirmed breast cancer cases.

Results and Conclusions: None yet.

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, Soda M (EN), Neta G, Brenner A, Berrington de Gonzalez A, Mabuchi K, Preston DL, Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and the incidence of soft tissue and bone tumors based on histologically reviewed diagnoses, and to clarify the histological characteristics of radiation-induced soft tissue and bone tumors.

Background and Significance: Sarcomas of the soft tissues and bones are known to be associated with high therapeutic doses of radiation. The broad classification of “sarcoma” showed a suggestive association with radiation in the recent LSS tumor incidence report. This study will provide more definitive evidence regarding the effects of radiation on histologically reviewed soft tissues and bone tumors.

Study Methods: Potential cases of soft tissue and bone tumors collected from cancer registries and other available sources will be histologically reviewed by pathologists.

Study Progress: A total of 88 cases with malignant tumor of soft tissue and bone were histologically confirmed among 127 potential cases collected from collaborating hospitals. They were derived from 160 cases that were accepted by

screening information sheets of 4,381 possible cases. Data analysis is being conducted in collaboration with U.S. NCI.

Results and Conclusions: None yet.

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

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

Objectives: To clarify the relationship between radiation and uterine cancer incidence based on histologically reviewed diagnoses, and to clarify the histological characteristics of uterine cervix and corpus cancer associated with radiation.

Background and Significance: In the recent cancer incidence analyses, there was some suggestion that radiation may be associated with uterine corpus cancer, especially in subjects exposed at young ages, but no association was seen for cervical cancer. Other factors in the pathogenesis of cervical and corpus cancer include HPV infection and estrogens, respectively.

Study Methods: Uterine corpus cancer is given priority in this study. Potential corpus cancer cases collected from cancer registries and other available sources will be histologically reviewed by pathologists.

Study Progress: The first screening of case information sheets for a total of 1,592 possible cases of uterine corpus cancer has been completed and 381 cases were accepted for histological review. Collection of histological specimens for pathological review is being scheduled.

Results and Conclusions: None yet.

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

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

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 obtained before diagnosis. Enrollees included about 300 gastric 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 the intestinal type of gastric cancer, after adjusting for these risk factors (*Cancer Epidemiol Biomarkers Prev* 2007; 16:1224–8). The *LTA 252* genotype is associated with noncardiac gastric cancer of the diffuse type in Japan, and the genotype was an effect modifier for radiation dose (*Helicobacter* 2009; 14:571–9). Radiation risk was significant only for people without chronic gastritis in developing diffuse type noncardiac 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, Cologne JB (S), Ueda K (CH), Akahoshi M (CN), Ozasa K (EH), Chayama K

Objectives: 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.

Background and Significance: An increased risk of liver cancer with radiation dose has been reported based on mortality studies and tumor registries among A-bomb survivors, but hepatitis virus infection status was not taken into account. Other previous studies at RERF provided suggestive evidence of a possible interaction, i.e., they reported supermultiplicative effects between radiation exposure and chronic HCV infection in the etiology of HCC without cirrhosis. These questions are especially important because they may help explain differences in the magnitude of radiation-HCC risk seen in this population vs. in western populations where HCV infection is uncommon.

Study Methods: The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, time and method of serum storage, and counter-matched on radiation dose.

Study Progress: Development of statistical methods is underway to assess the joint effects of radiation and intermediate risk factors such as hepatitis virus infection in nested case-control studies. A paper on serum inflammatory markers associated with HCC risk has been submitted to an international journal.

Results and Conclusions: After adjusting for alcohol consumption, smoking habit, and body mass index (BMI), the RR (95% CI) at 1 Gy of radiation exposure for HCC, was 1.67 (1.22–2.35), while the RRs for HBV or HCV infection were 63 (20–241) and 83 (36–231), respectively. Those estimates changed little when radiation and viral effects were fit simultaneously. The ERR (95% CI) at 1 Gy for non-B, non-C HCC was 2.74 (1.26–7.04) with adjustment for alcohol consumption, smoking habit, or BMI. These results indicated that radiation exposure is associated independently with increased HCC risk, and that radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, smoking habit, or BMI (*Hepatology* 2011; 53:1237–45).

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

Ohishi W (CH), Neriishi K, Grant EJ (EH), Cologne JB (S), Sharp GB, Eguchi H, Nakachi K, Nakashima E (S), Izumi S, Fujiwara S, Akahoshi M (CN), Key TJ, Stevens RG, Berrington de Gonzalez A

Objectives: To characterize the joint effects of radiation and serum-based indicators of hormonal status, oxidative stress, and phytoestrogen consumption in breast and endometrial cancer development.

Background and Significance: It is not known to what extent known breast-cancer risk factors are directly or indirectly involved in radiation-induced cancer. Assessing the joint effects of radiation and other factors using complex causal models may shed light on the mechanisms of radiogenic breast and endometrial cancer etiology.

Study Methods: Serum measurement from the 243 breast cancer cases whose blood samples were collected up to 30 years before cancer diagnosis and 486 age-matched controls, selected with counter-matching on radiation dose, were conducted in 2007–2008. Statistical analyses and manuscript preparations are under way.

Study Progress: Manuscripts were published on breast cancer risk related to radiation exposure and ferritin levels and on hormone levels in relation to radiation dose. A manuscript on radiation risk for breast cancer with hormone levels as a confounder or effect-modifier is under preparation. Data have been released for use by an international breast cancer consortium.

Results and Conclusions: Serum levels of estradiol and testosterone increased with radiation dose in cancer-free postmenopausal women (*Radiation Res* 2011; 176:678–87). The radiation-unadjusted and -adjusted relative risks (95% CI) of breast cancer for a 1 log unit increase in ferritin were 1.4 (1.1–1.8) and 1.3 (1.0–1.7), respectively. The mechanism of the joint effects of ferritin and radiation on postmenopausal breast cancer risk could not be assessed (*Cancer Sci* 2011; 102:2236–40).

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)

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

Objectives: To clarify the relationship between radiation and skin cancer incidence based on histologically reviewed diagnosis, and to clarify the histological characteristics of radiation-induced skin cancer in the Life Span Study (LSS) cohort.

Background and Significance: It has been reported that skin cancer incidence, especially nonmelanoma skin cancer, increased with radiation exposure among patients with radiotherapy and atomic bomb survivors. The effects of radiation upon skin cancer have been reported to have a long latency period among atomic bomb survivors. RP 2-02 study is a 10-year extension of RP 2-91, which showed an

excess of basal cell carcinoma (n = 80) during the period from 1950 to 1987.

Study Methods: Potential skin tumor cases collected from cancer registries and other available sources were histologically reviewed by pathologists.

Study Progress: We confirmed a total of 336 cases with first primary skin cancer (123 basal cell carcinoma, 178 squamous cell carcinoma including *in situ* cancer, and others), adding 128 new cases to the previous 1958–1986 series. Analysis has been completed and a manuscript submitted.

Results and Conclusion: ERRs were estimated assuming a linear dose response, and only basal cell carcinoma had a statistically significant positive dose response. However, the basal cell carcinoma data fit a threshold model better than a linear model, and the dose threshold was estimated to be 0.63 Gy (95% CI: 0.30, 0.90).

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

Fujihara M, Namba K, Tokunaga M, Takahara O, Soda M (EN), Dohy H, Kamada N, Tomonaga M, Preston DL, Mabuchi K, Sakata R (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and lymphoid malignancy incidence based on histologically reviewed diagnoses, and to clarify the histological characteristics of radiation-induced lymphoid malignancies.

Background and Significance: There have been controversial findings on the effects of radiation on lymphoid malignancies. It is necessary to thoroughly analyze the data on lymphoid malignancies in the A-bomb survivors through histological reviews based on current standardized diagnostic criteria.

Study Methods: Potential lymphoid malignancies collected from various available sources including cancer registries and others are histologically reviewed by pathologists.

Study Progress: A total of 476 definite and probable cases of malignant lymphomas (ML) from the period of 1950–1995 were identified for analyses. They included 140 cases of diffuse large B-cell lymphoma (DLBCL), 70 of multiple myeloma, 96 of other B-cell lymphoma, 39 of adult T-cell leukemia (ATL), 30 of peripheral T-cell lymphoma (PTCL), 61 of other T-cell lymphoma, 13 of Hodgkin lymphoma, 24 of other non-Hodgkin lymphoma, and 3 of acute lymphocytic leukemia (ALL). Analysis of radiation risks for specific histological types of malignant lymphoma and pathological overviews are being conducted.

Results and Conclusions: None yet.

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

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

Objectives: To quantify the risk of lung cancer and the variation in histological subtypes with special interest in possible interactions with smoking.

Background and Significance: Radiation-related risks of lung cancer were observed in the latest tumor registry-based incidence and mortality reports. However, several specific questions are being addressed, including the

specificity of various cell types involved in radiation- versus smoking-related cancers, confounding and joint effects of smoking and radiation exposure.

Study Methods: A pathologist panel histologically confirmed 2,368 lung cancers among the 5,711 potential cases ascertained from cancer registries and other available sources between 1958 and 1999. The data on smoking habits were collected from a series of questionnaire surveys on the LSS and AHS conducted between 1965–1991.

Study Progress: The second paper on the joint effects of smoking and radiation on lung cancer by specific histological type was published (Egawa H et al., *Radiat Res* 2012; 178(3):191–201).

Results and Conclusions: Both smoking and radiation exposure appeared to significantly increase the risk of each of the major lung cancer histologic types. While the main effects of smoking and radiation varied in magnitude among the types, there appeared to be the possibility of a common pattern of the interaction between smoking and radiation exposure; the smoking-adjusted radiation effect among smokers tended to be larger for moderate smokers than for heavier smokers.

RP 2-92 Studies on ovarian tumor incidence among the RERF Extended Life Span Study cohort

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

Objectives: To clarify the relationship between radiation and ovarian tumor incidence based on histologically reviewed diagnoses.

Background and Significance: An increased risk of ovarian cancer among atomic-bomb survivors has been known, but there was no clear evidence of which specific histologic types were associated with radiation exposure.

Study Methods: Potential ovarian tumor cases collected from cancer registries and other available sources were histologically reviewed by pathologists from 1950 to 1987.

Study Progress: All work related to case identification and histological verification was completed. A total of 601 ovarian tumors (182 malignant, 419 benign tumors) were histologically confirmed. A case-series report was published. A paper on radiation and population-based incidence is in preparation.

Results and Conclusions: Serous epithelial tumor was the most frequent histological type in both malignant and benign tumors, then mucinous epithelial tumors, sex-cord stromal tumors, and germ cell tumors. Malignant common epithelial tumors had a nominally high ERR/Gy, but not significant. Among them, mucinous tumors had a weaker association than the other subtypes. There was a significant apparent survival advantage for those with the malignant mucinous type compared with the serous type. Within tumor types, there was no consistent pattern of survival by radiation dose.

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

RP 5-11 Extension of study period (to 2005) and amendment of review procedures of the “Studies on thyroid tumor incidence among the RERF Extended Life Span Study cohort, 1950–87” (Addendum to RP 6-91)

Ozasa K (EH), Hayashi Y, Tsuda N, Tokunaga M, Yonehara S, Ito M, Sekine I, Neta G, Brenner A, Mabuchi K, Preston DL, Furukawa K (S), Imaizumi M (CN), Kodama K (CS)

Objectives: To clarify the shape of the radiation dose-response relationships for malignant and benign tumors, variations in risk by modifiers, and the relative importance of different histological subtypes to radiation exposure based on histo-pathologically verified thyroid tumor cases.

Background and Significance: Thyroid cancer was one of the earliest solid cancers found to be increased among atomic-bomb survivors and other irradiated cohorts. This study includes the 1958–2005 period of case ascertainment for both benign and malignant thyroid tumor cases.

Study Methods: Potential thyroid tumor cases collected from cancer registries and other available sources were histologically reviewed by pathologists.

Study Progress: A total of 371 cases of thyroid cancer were histologically confirmed by pathologist from the period of 1958–2005. A detailed analysis using information on lifestyle factors is being conducted in collaboration with the U.S. NCI. A paper was published in collaboration with the Department of Statistics (Furukawa K et al., *Int J Cancer* 2013; 132(5):1222–6).

Results and Conclusions: A linear dose-response model fit the data well. The excess relative risk of thyroid cancer at 1 Gy of radiation exposure was estimated as 1.28 (95% confidence interval: 0.59–2.70) at age 60 after acute exposure at age 10. The risk decreased sharply with increasing age at exposure and there was little evidence of increased thyroid cancer rates for those exposed after age 20. Among those exposed as children or adolescents, the attributable fraction decreased with attained age, but remained elevated (16%, 95% CI: 6.24) during the latest follow-up period of 1996–2005.

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

Ozasa K (EH), Sekine I, Soda M (EN), Tokunaga M, Mabuchi K, Cullings HM (S), Kodama K (CS)

Objectives: To establish guidelines for pathological studies on the incidence of specific cancers in the LSS population. Guidelines include specifying basic study designs, methodology, and procedures to maintain uniformity. These guidelines are intended to simplify the preparation of subsequent research plans for site-specific cancer incidence studies and simultaneously provide for uniformity in basic design and operation of the studies.

Background and Significance: Some uncertainties have been detected in the accuracy of diagnoses based on death certificates or tumor and tissue registries in the LSS cohort studies. Standardized pathological reviews are therefore valuable. These guidelines are intended to simplify the preparation of subsequent research plans for site-specific

cancer incidence studies and to provide uniformity in the basic design and conduct of studies.

Study Methods: The framework for the site-specific cancer studies includes three major areas: case ascertainment, pathological review, and data analysis.

Study Progress: Research protocols have been written for cancers and/or tumors of the liver (RP 5-90), salivary glands (RP 1-91), skin (RP 2-91, updated to RP 2-02), ovary (RP 2-92), thyroid (RP 6-91), central nervous system (RP 4-92), breast (RP 6-93, updated to RP 5-08, and RP 6-10 for intrinsic subtypes), lung (RP 1-94), lymphoid system (RP 3-94), uterus (RP 1-06), and soft tissue and bone (RP 4-07). Among them, studies of the liver (RP 5-90), salivary gland (RP 1-91), and central nervous system (RP 4-92) have been completed and others are underway.

Results and Conclusions: Essential methods for site-specific cancer incidence studies with histology reviews were established.

RP 29-60 Detection of leukemia and related disorders

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

Objectives: The purpose of the study is to determine the incidence and risks of leukemia and related hematological disorders in the radiation-exposed persons of Hiroshima and Nagasaki. Primary questions are those concerning the leukemogenic effects of ionizing radiation.

Background and Significance: A significant excess risk of radiation-induced leukemia was observed within five years after the bombings, and the latest data suggest the possibility of a small, continued excess of leukemia and an increased risk of myelodysplastic syndrome (MDS). Leukemia was specifically surveyed in the Leukemia Registry (TR 5-65) by ABCC from 1946 to the early 1990s and have been reclassified using a modern leukemia classification system. Currently leukemia cases are collected through usual population-based cancer registries. Therefore, leukemia has been more completely surveyed than other malignancies.

Study Methods: Incident cases of leukemia and related hematological disorders have been collected through the Leukemia Registries and the population-based cancer registries in both cities.

Study Progress: Analyses to clarify the relation between radiation dose and the incidence of leukemia and related hematological disorders are being conducted in collaboration with the Statistics Department. A paper has been accepted for publication in *Radiation Research*.

Results and Conclusion: There was a non-linear dose response for leukemias (excluding chronic lymphocytic leukemia and adult T-cell leukemia) which varied markedly with time and age at exposure, with much of the evidence for this non-linearity arising from the acute myeloid leukemia risks. Although the leukemia excess risks generally declined with attained age or time since exposure, there was evidence that the radiation-associated excess leukemia risks, especially for acute myeloid leukemia, had persisted throughout the follow-up period out to 55 years after the bombings. As in earlier analyses, there was a weak suggestion of a radiation

dose response for non-Hodgkin lymphoma among men with no indication of such an effect among women. There was no evidence of radiation-associated excess risks for either Hodgkin lymphoma or multiple myeloma.

RP-A5-12 A proposal to join the diet and bladder cancer pooling project

Grant EJ (EH), Ozasa K (EH), Ohishi W (CH), Akahoshi M (CN)

Objectives: The Diet and Bladder Cancer Pooling Project (DBCP) is a collaborative pooling project designed to investigate the association of diet and bladder cancer using data from bladder cancer studies that have been conducted around the world. The Principal Investigator of the project is Dr. Maurice Zeegers of Maastricht University (formerly of Cambridge University).

Background and Significance: Bladder cancer is the most expensive malignancy to treat from diagnosis until death ranging from 96,000–187,000 U.S. dollars per patient. With 400,000 new patients occurring yearly worldwide, it is the 7th most common cancer. As is the case for many tumors, the development of bladder cancer is likely to be influenced by diet. However, the role of diet in bladder cancer could be more pronounced, as the bladder is an excretion organ. Previous research has suggested that 30% of all bladder cancers could have been prevented by dietary modifications. However, it is not yet clear which specific foods or nutrients are involved and therefore which dietary modifications should be recommended to prevent this disease. Efforts towards the prevention of this disease by dietary recommendations could directly lead to a substantial reduction of morbidity, mortality, and healthcare costs. A 30% less common bladder cancer in the USA, for example, would equal a reduction of 1.2 billion U.S. dollars of total medical care expenditures per year.

Study Methods: Pool data from many different studies. Data will need to be harmonized as a first step. Later, pooled analyses will be performed.

Study Progress: As of 2011, the DBCP had recruited 18 studies from the United States, Belgium, the Netherlands, Sweden, Italy, Germany, France, United Kingdom, Hungary, Romania, Slovakia, Spain, Singapore, and China representing more than 30,000 controls and 10,000 bladder cancer cases. RERF data were sent to Dr. Zeegers in October 2012. Currently harmonizing procedures are taking place.

Results and Conclusions: None at this early point in the project.

RP-A3-11 A proposal to join the Asia Cohort Consortium. Project 2: BMI and total mortality (Phase II), Project 3: Body mass index and risk of intermediate and low rate cancers in Asian populations, Project 4: Diet and mortality among Asians

Grant EJ (EH), Ozasa K (EH), Ohishi W (CH), Akahoshi M (CN), Shore RE (D)

Objectives: This project builds upon the successful introduction of RP-A3-10, which established the collaboration between RERF and the Asia Cohort Consortium (ACC). This RP expands the data shared (additional cancer types and additional lifestyle data). New projects include another BMI and mortality study, a BMI and cancer incidence study, and

a diet study.

Background and Significance: The ACC seeks to understand the relationship between environmental exposures and the etiology of disease through a newly established cohort of over one million healthy Asians who are followed for various disease endpoints, including cancer. Investigators from China, India, Japan, Korea, Malaysia, Singapore, Taiwan, and Bangladesh are included.

Study Methods: The Fred Hutchinson Cancer Research Center in Seattle, Washington, USA acts as the data coordinator for this multi-cohort study. Analyses use pooled data analyses for cohort data to derive risk estimates of various exposures.

Study Progress: This RP was approved in 2011. Additional data to support this RP have been uploaded to the project's coordinating system and harmonized with the other data. Weekly teleconferences and annual meetings provide the communication needed to move projects ahead. Current efforts are focused on a follow-up paper on BMI and overall mortality, which was published in 2011 (Zheng et al., *N Engl J Med* 2011; 364(8):719–29). The follow-up paper will focus primarily on smoking, trying to generate population attributable risk estimates for all of Asia. A second project was to write an NIH grant to support research on rare cancers, but the proposal was rejected without review. A paper on BMI and pancreatic cancer was published. A paper on dietary intake and overall mortality has been drafted and is currently being circulated.

Results and Conclusions: Unpublished (preliminary) results show that the overall mortality risk of ever smokers was about 50% higher than never smokers. Direct tobacco smoking (i.e., not second-hand) accounted for 25% of all male deaths or 2.1 million deaths in the seven countries studied (Bangladesh, India, mainland China, Japan, Singapore, South Korea, and Taiwan—nearly 75% of Asia's total population). Another analysis found no statistically significant overall association between BMI and the risk of death from pancreas cancer in all Asians, and obesity was unrelated to the risk of mortality in both East Asians and South Asians. In the meat analyses, preliminary results suggest there was no association of increased overall mortality due to increased meat consumption. A big challenge in the diet data is assessing the possible impact of reverse causation due to socio-economic factors (i.e., higher income leads to more meat consumption but also better overall nutrition and medical care).

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), Hsu WL (S), Grant EJ (EH), Ohishi W (CH), Fujiwara S, Cullings HM (S)

Objectives: Extend regression methods for assessing mediation to nested case-control data that allow valid estimation of radiation risk and estimation of the amount of risk attributable to individual causal paths (especially mediation of radiation risk).

Background and Significance: Mediation of radiation risk by disease risk factors causally affected by radiation (e.g., radiation → HBV infection susceptibility → hepatocellular carcinoma; radiation → inflammation → heart disease)

has not been adequately explored in the atomic-bomb survivor studies. Standard regression models produce risk parameters that cannot be interpreted in terms of mediation. Regression models for assessing complex causal mechanisms are not immediately applicable to the nested case-control study design, used in the RERF studies of liver cancer (RP 1-04), gastric cancer (RP 2-04), and breast cancer (RP 6-02), or to the case-cohort study design used in the RERF cancer and immunogenome study (RP 4-04).

Study Methods: We will apply a combination of statistical theory, computer simulation, and comparative analyses using actual data. Methods will be evaluated using cohort data from the AHS follow-up study of hepatitis virus and liver disease (RP 9-92) and by computer simulation.

Study Progress: An ad hoc path analysis approach has been applied to existing nested case-control data on liver cancer and breast cancer, and a manuscript is soon to be completed describing joint effects of radiation and serum sex hormones in the case of breast cancer. A thorough literature review has been completed and a review paper is in preparation.

Results and Conclusions: There is a major distinction between biological mediation in principle and practical mediation in fact. Although the theoretical requirements for mediation are satisfied with certain serum sex hormones and growth factors in the case of breast cancer and with HBV in the case of liver cancer, there is no evidence of practical mediation in either study.

Owing to natural variation and differences in magnitude of risk, potential mediators that are strong risk factors may not evidence a great deal of mediation of radiation risk despite highly significant dose responses.

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, Ohishi W (CH), Akahoshi M (CN), Shore RE (D)

Objectives: This study creates a framework by which RERF becomes a member of the Asia Cohort Consortium (ACC), a multi-centered project with a combined cohort of over 1 million Asians with lifestyle, cancer incidence, and mortality data. In addition, this RP initiated RERF's first collaborative project with the ACC entitled: *Tobacco smoking, alcohol drinking, body mass index and risk of rare cancers*.

Background and Significance: Current knowledge on the causes of rare neoplasms (i.e., neoplasms with age-adjusted incidence rate below 1.0/100,000 in most human populations) is hampered by the difficulty to assemble a large enough set of cases to study even potent carcinogens with high prevalence of exposure, such as tobacco smoking. Investigators from China, India, Japan, Korea, Malaysia, Singapore, Taiwan, and other countries have combined their cohorts to create a pooled analysis cohort of over 1 million persons to address these and other questions relating to lifestyle and the etiology of disease among Asians.

Study Methods: The project will require the integration of data on exposure of interest, covariates, and outcome among the participating cohorts. The coordinating center is the Fred Hutchinson Cancer Research Center in Seattle,

Washington, USA. RERF data have been restricted to persons with less than 100 mGy shielded kerma exposure and to persons who participated in at least one lifestyle survey. Approximately 53,000 persons are included in the data contributed by RERF. Pooled methods for cohort data will be employed in the analyses.

Study Progress: RERF researchers have attended meetings, weekly telephone conferences and participated in manuscript preparation. Data have been harmonized and a paper has been published. This RP provides a foundation for additional research questions asked by the ACC and another RP has been written to expand the scope of the collaboration.

Results and Conclusions: For a study of small intestine cancers, we harmonized the data across 12 cohort studies from mainland China, Japan, Korea, Singapore, and Taiwan, comprising over 500,000 subjects followed for an average of 10.6 years. A total of 134 incident cases of small intestine cancer were observed (49 adenocarcinoma, 11 carcinoid, 46 other histologic types, and 28 of unknown histology). There was a statistically non-significant trend towards an increased hazard ratio (HR) in subjects with high BMI (HR for BMI greater than 27.5 kg/m², compared to 22.6–25.0, 1.50; 95% confidence interval [CI] 0.76, 2.96). No association was suggested for tobacco smoking; men drinking more than 400 g of ethanol per week had an HR of 1.57 (95% CI 0.66, 3.70), compared to abstainers. This study supports the hypothesis that elevated BMI may be a risk factor for small intestine cancer. An etiologic role of alcohol drinking was suggested. The results reinforce the existing evidence that the epidemiology of small intestine cancer resembles that of colorectal cancer.

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

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

Objectives: To improve our understanding of radiogenic thyroid cancer and probe additional questions that remain regarding risk of radiation-related thyroid cancer associated with juvenile or adult exposure.

Background and Significance: A causal relationship between ionizing radiation and thyroid neoplasia has been well established. The association for thyroid cancer was first identified among A-bomb survivors in 1963 (Socolow et al., *N Engl J Med* 1963). However, data have been limited with regard to several important radiation risk issues: the slope of the dose-response curve, dose fractionation, age at exposure, length of time since exposure, host susceptibility, and histologic cell type. The original pooled analysis (Ron E et al., *Radiat Res* 1995; 141:259–77) of the risk of developing thyroid cancer after external radiation exposure was based on seven major epidemiologic studies. A new, pooled analysis based on considerably more data will significantly add to what is known about radiation-related thyroid cancer and its modifying factors.

Study Methods: An updated and expanded pooled analysis of 16 studies of radiation exposure and thyroid cancer risk will be carried out. To carry out the Poisson regression analyses, data were cross-classified by attained age, age at exposure, calendar period, study population, dose, and other variables of interest.

Study Progress: A paper on thyroid cancer incidence following radiotherapy for childhood cancer was published in *Radiation Research*, and an analysis including the A-bomb study and other exposed populations is underway.

Results and Conclusions: The following results were obtained from the analysis of thyroid cancer following radiotherapy for childhood cancer. Dose-related relative risks increased approximately linearly up to about 10 Gy, then leveled off. The fitted RR at 10 Gy was 13.7 (95% confidence interval: 8.0–24.0). Dose-related excess RRs increased with decreasing age at exposure ($p < 0.01$), but did not vary with attained age or time since exposure, remaining elevated 25+ years after exposure.

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

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

Objectives: To determine the population-based temporal trends in esophageal and gastric cancer incidence in Hiroshima and Nagasaki by histopathology and anatomic site. To assess the radiation effects on esophageal and gastric cancer by histopathology and anatomic site. To identify which modifiable risk factors have the greatest public health impact on esophageal and gastric cancers in the LSS.

Background and Significance: Over the last several decades, incidence rates for esophageal cancer have varied, while incidence rates for stomach cancer have generally decreased in many countries. Prior work has suggested that these trends likely reflect patterns in certain cancer subcategories. Knowing the temporal patterns in Asian populations could provide important insights into the etiology of esophageal and gastric cancers. The LSS data have shown that the risks for esophageal and gastric cancers are significantly increased by radiation (Preston et al., *Radiat Res* 2007; 168:1–64). However, associations by anatomic location and histological cell type have not been specifically examined. Esophageal and gastric cancers, like other aerodigestive malignancies, have several risk factors in common: smoking, drinking alcohol, consuming low levels of fruits and vegetables, gastroesophageal reflux disease, and being overweight. However, among Asian populations, including those in Japan, it is not clear what proportion of esophageal and gastric cancers are due to these modifiable lifestyle factors. Differences in risk factor distributions may lead to differences in the population attributable risks (PAR) and, consequently, the public health impact of these factors.

Study Methods: For the temporal trend analysis, data from the population-based tumor registries of Hiroshima and Nagasaki were used. For the radiation effects and risk factor analyses, the LSS cohort data were used, analyzing sex, histopathology and location, age at exposure, attained age, and time since exposure. In the risk factor analysis, the main predictors were tobacco smoking, alcohol consumption, body mass index (BMI), and dietary patterns. The dietary data analysis emphasized the consumption of fruits and vegetables, which has been shown to lower the risk for

esophageal/gastric cancer in prior studies. To assess the potential public health impact of several modifiable risk factors, PARs for tobacco, alcohol, BMI, and dietary patterns will be estimated, using a method that allows for the determination of partial PARs for the modifiable risk factors, while controlling for the non-modifiable risk factors such as age and sex.

Study Progress: The study was dormant for several years but restarted in mid-2012. All analyses have been completed and two manuscripts were written in 2012 and have been circulated.

Results and Conclusions: For the population-based study: esophageal cancer increased for males and remained stable for females. Gastric cancer decreased for both males and females, mainly due to decreases in intestinal-type cancers. Gastric cancer early diagnosis increased in parallel with decreases in the death certificate only (DCO) fraction, especially for males; which may suggest lower screening rates for females. For the LSS-based radiation study: the lower anatomic segment showed the highest dose response (HR = 2.5) for esophageal cancer. For gastric cancer, diffuse cases showed the highest dose response (HR = 1.9). Smoking was shown to be the most important modifiable risk factor. Temporal trends in esophageal and gastric cancer rates were characterized. High radiation risks were observed in certain subtypes of these cancers. The results indicate that population risk reduction can be effected by avoiding smoking, by drinking alcohol and consuming hot/salty food only in moderation, and by increasing consumption of chicken, tofu, and fruit.

Special Cancer Studies Publications

RERF Reports (RR)

◆ Egawa H, Furukawa K, Preston D, Funamoto S, Yonehara S, Matsuo T, Tokuoka S, Suyama A, Ozasa K, Kodama K, Mabuchi K: Radiation and smoking effects on lung cancer incidence by histological types among atomic bomb survivors. *Radiat Res* 2012 (September); 178(3):191–201. (RR 11-11) © 2012 by Radiation Research Society

[Abstract] While the risk of lung cancer associated separately with smoking and radiation exposure has been widely reported, it is not clear how smoking and radiation together contribute to the risk of specific lung cancer histological types. With individual smoking histories and radiation dose estimates, we characterized the joint effects of radiation and smoking on type-specific lung cancer rates among the Life Span Study cohort of Japanese atomic bomb survivors. Among 105,404 cohort subjects followed between 1958 and 1999, 1,803 first primary lung cancer incident cases were diagnosed and classified by histological type. Poisson regression methods were used to estimate excess relative risks under several interaction models.

Adenocarcinoma (636 cases), squamous-cell carcinoma (330) and small-cell carcinoma (194) made up 90% of the cases with known histology. Both smoking and radiation exposure significantly increased the risk of each major lung cancer histological type. Smoking-associated excess relative risks were significantly larger for small-cell and squamous-cell carcinomas than for adenocarcinoma. The gender-averaged excess relative risks per 1 Gy of radiation

(for never-smokers at age 70 after radiation exposure at age 30) were estimated as 1.49 (95% confidence interval 0.1–4.6) for small-cell carcinoma, 0.75 (0.3–1.3) for adenocarcinoma, and 0.27 (0–1.5) for squamous-cell carcinoma. Under a model allowing radiation effects to vary with levels of smoking, the nature of the joint effect of smoking and radiation showed a similar pattern for different histological types in which the radiation-associated excess relative risk tended to be larger for moderate smokers than for heavy smokers. However, in contrast to analyses of all lung cancers as a group, such complicated interactions did not describe the data significantly better than either simple additive or multiplicative interaction models for any of the type-specific analyses.

◆ Furukawa K, Preston D, Funamoto S, Yonehara S, Ito M, Tokuoka S, Sugiyama H, Soda M, Ozasa K, Mabuchi K: Long-term trend of thyroid cancer risk among Japanese atomic-bomb survivors: 60 years after exposure. *Int J Cancer* 2013 (March); 132(5):1222–6. (RR 5-12) © 2012 UICC (*This abstract was reprinted by permission of John Wiley & Sons, Inc.*)

[Abstract] Thyroid cancer risk following exposure to ionizing radiation in childhood and adolescence is a topic of public concern. To characterize the long-term temporal trend and age-at-exposure variation in the radiation-induced risk of thyroid cancer, we analyzed thyroid cancer incidence data for the period from 1958 through 2005 among 105,401 members of the Life Span Study cohort of Japanese atomic-bomb survivors. During the follow-up period, 371 thyroid cancer cases (excluding those with microcarcinoma with a diameter <10 mm) were identified as a first primary among the eligible subjects. Using a linear dose-response model, the excess relative risk of thyroid cancer at 1 Gy of radiation exposure was estimated as 1.28 (95% confidence interval: 0.59–2.70) at age 60 after acute exposure at age 10. The risk decreased sharply with increasing age-at-exposure and there was little evidence of increased thyroid cancer rates for those exposed after age 20. About 36% of the thyroid cancer cases among those exposed before age 20 were estimated to be attributable to radiation exposure. While the magnitude of the excess risk has decreased with increasing attained age or time since exposure, the excess thyroid cancer risk associated with childhood exposure has persisted for >50 years after exposure.

◆ Hsu WL, Preston DL, Soda M, Sugiyama H, Funamoto S, Kodama K, Kimura A, Kamada N, Dohy H, Tomonaga M, Iwanaga M, Miyazaki Y, Cullings HM, Suyama A, Ozasa K, Shore RE, Mabuchi K: The incidence of leukemia, lymphoma and multiple myeloma among atomic bomb survivors: 1950–2001. *Radiat Res* 2013 (March); 179(3):361–82. (RR 29-11) © 2013 by Radiation Research Society

[Abstract] A marked increase in leukemia risks was the first and most striking late effect of radiation exposure seen among the Hiroshima and Nagasaki atomic bomb survivors. This article presents analyses of radiation effects on leukemia, lymphoma and multiple myeloma incidence in the Life Span Study cohort of atomic bomb survivors updated 14 years since the last comprehensive report on these malignancies. These analyses make use

of tumor- and leukemia-registry based incidence data on 113,011 cohort members with 3.6 million person-years of follow-up from late 1950 through the end of 2001. In addition to a detailed analysis of the excess risk for all leukemias other than chronic lymphocytic leukemia or adult T-cell leukemia (neither of which appear to be radiation-related), we present results for the major hematopoietic malignancy types: acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, adult T-cell leukemia, Hodgkin and non-Hodgkin lymphoma and multiple myeloma. Poisson regression methods were used to characterize the shape of the radiation dose-response relationship and, to the extent the data allowed, to investigate variation in the excess risks with gender, attained age, exposure age and time since exposure. In contrast to the previous report that focused on describing excess absolute rates, we considered both excess absolute rate (EAR) and excess relative risk (ERR) models and found that ERR models can often provide equivalent and sometimes more parsimonious descriptions of the excess risk than EAR models. The leukemia results indicated that there was a nonlinear dose response for leukemias other than chronic lymphocytic leukemia or adult T-cell leukemia, which varied markedly with time and age at exposure, with much of the evidence for this nonlinearity arising from the acute myeloid leukemia risks. Although the leukemia excess risks generally declined with attained age or time since exposure, there was evidence that the radiation-associated excess leukemia risks, especially for acute myeloid leukemia, had persisted throughout the follow-up period out to 55 years after the bombings. As in earlier analyses, there was a weak suggestion of a radiation dose response for non-Hodgkin lymphoma among men, with no indication of such an effect among women. There was no evidence of radiation-associated excess risks for either Hodgkin lymphoma or multiple myeloma.

Other Journal Publications

◆ Cologne JB: Commentary on “Development of a prediction model for 10-year risk of hepatocellular carcinoma: The Japan Public Health Center-based Prospective Study Cohort II” by Michikawa et al. *Prevent Med* 2012 (August); 55(2):144–5.

◆ Kodama K, Ozasa K, Katayama H, Shore RE, Okubo T: Radiation effects on cancer risks in the Life Span Study cohort. *Radiat Prot Dosimetry* 2012 (October); 151(4):674–6. (related to *Life Span Study* and *Tumor and Tissue Registries*)

◆ Ohishi W, Chayama K: Risk factors for non-B, C hepatocellular carcinoma. *Rinsho Shokaki Naika [Clin Gastroenterol]* 2012 (May); 27(5): 587–93. (Japanese)

Manuscript in Press

✂ Lin Y, Fu R, Grant E, Chen Y, Lee JE, Gupta PC, Ramadas K, Inoue M, Tsugane S, Gao YT, Tamakoshi A, Shu XO, Ozasa K, Tsuji I, Kakizaki M, Tanaka H, Chen CJ, Yoo KY, Ahn YO, Ahsan H, Pednekar MS, Sauvaget C, Sasazuki S, Yang G, Xiang YB, Ohishi W, Watanabe T, Nishino Y, Matsuo K, You SL, Park SK, Kim DH, Parvez F, Rolland B, McLerran D, Sinha R, Boffetta P, Zheng W, Thornquist M, Feng Z,

Kang D, Potter JD: Association of body mass index and risk of death from pancreas cancer in Asians: findings from the Asia Cohort Consortium. *Eur J Cancer Prevent*.

Special Cancer Studies Oral Presentations

❖ Ohishi W, Cologne JB, Fujiwara S, Akahoshi M, Tsuge M, Chayama K. Study of inflammatory markers contributing to risk of hepatocellular carcinoma: a nested case-control study. 48th Annual Meeting of the Japan Society of Hepatology, 7–8 June 2012, Kanazawa

❖ Furukawa K. Thyroid cancer incidence among atomic-bomb survivors: An update in the Life Span Study. ASA (American Statistical Association) Conference on Radiation and Health, 10–13 June 2012, Kennebunkport, Maine, USA

❖ Misumi M, Sugiyama H, Ozasa K, Cullings HM. Effect of dose uncertainty on estimated threshold of skin cancer dose-response in the Radiation Effects Research Foundation Life Span Study cohort. 2012 ASA (American Statistical Association) Conference on Radiation and Health, 10–13 June 2012, Kennebunkport, Maine, USA

❖ Furukawa K, Preston DL, Funamoto S, Egawa H, Ozasa K. Radiation, smoking and lung cancer: Epidemiological findings among atomic-bomb survivors. 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima

❖ Sugiyama H, Misumi M, Soda M, Tokuoka S, Grant EJ, Sakata R, Ozasa K. Radiation risk of skin cancer incidence among atomic-bomb survivors in Hiroshima and Nagasaki, 1958–1996. 35th Annual Meeting of the Japanese Society of Cancer Epidemiology, 5–6 July 2012, Hiroshima

❖ Misumi M, Sugiyama H, Grant EJ, Sakata R, Ozasa K, Cullings HM. Radiation dose-response of skin cancer risk in the Radiation Effects Research Foundation Life Span Study cohort. 26th International Biometric Conference, 26–31 August 2012, Kobe

❖ Grant EJ, Cologne JB, Sharp GB, Stevens RG, Nakachi K, Land CE, Berrington de Gonzalez A, Neriishi K. Radiation risk of breast cancer after adjustment for serum hormone levels among atomic-bomb survivors. 55th Annual Meeting of the Japan Radiation Research Society, 6–8 September 2012, Sendai

❖ Hsu WL, Preston DL, Cullings HM, Ozasa K, Shore RE, Mabuchi K. Incidence of leukemia, lymphoma, and multiple myeloma among the Japanese atomic-bomb survivors: 1950–2001. 58th Annual Meeting of the Radiation Research Society, 30 September–3 October 2012, San Juan, Puerto Rico

❖ Ohishi W, Cologne JB, Fujiwara S, Hayashi T, Niwa Y, Ueda K, Tsuge M, Chayama K. Inflammatory markers are associated with risk of hepatocellular carcinoma: A nested case-control study. 63rd Annual Meeting of the American Association for the Study of Liver Diseases, 8–14 November 2012, Boston, Massachusetts, USA

Research Protocol 18-61

Tumor and Tissue Registries, Hiroshima and Nagasaki

RP 18-61 Tumor and tissue registry study in Hiroshima and Nagasaki

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

Objectives: The purpose of the study is to determine the incidence and risks of all types of neoplasms in the exposed persons of Hiroshima and Nagasaki.

Background and Significance: Population-based tumor registries have been active in Hiroshima since 1957 and in Nagasaki since 1958. Tissue registry systems were established in Hiroshima in 1973 and in Nagasaki in 1974. ABCC-RERF has assisted in the collection of cases and the management and maintenance of those databases. The information and materials are available in the Epidemiology Department for incidence studies, special cancer studies, case-control studies, etc.

Study Methods: Information on tumor diagnosis is collected through: notifications from hospitals and other medical facilities, on-site abstraction of medical records by RERF personnel, and death certificates. Information on pathological diagnosis of tumor and materials is collected from hospitals and local pathology laboratories for the tissue registries. Every year the Epidemiology Department retrieves the information on RERF study subjects from the registries with permission of the registry authorities.

Study Progress: Case collection by notifications and death certificates has been nearly completed through 2011 in both Hiroshima and Nagasaki prefectures. On-site record abstraction is complete through 2009 in both Hiroshima city and Nagasaki prefecture. Tissue diagnoses and samples are being collected through 2010 in Hiroshima prefecture and 2008 in Nagasaki prefecture. The Department of Epidemiology is linking registry information with RERF subjects; this is a laborious process and is currently being performed through 2005.

Results and Conclusion: A number of scientific papers based on the tumor and tissue registry data have been published. Annual reports on incidence data for 2008 in both Hiroshima and Nagasaki prefectures were published. The cancer incidence information through 2005 has been cross-checked with the database of the LSS, *in utero*, and F₁, and summarized. The new project to report the radiation-related ERR and EAR for solid cancer incidence among LSS has been initiated and data have been distributed to collaborating authors for analysis.

Tumor and Tissue Registries Publications

RERF Reports (RR)

◆ Cologne JB, Hsu WL, Abbott RD, Ohishi W, Grant EJ, Fujiwara S, Cullings HM: Proportional hazards regression in epidemiologic follow-up studies: An intuitive consideration of primary time scale. *Epidemiology* 2012 (July); 23(4):565–73. (RR 12-11) (related to *Adult Health Study* and *Special Clinical Studies*) (refer to abstract in *Adult Health Study* Publications)

◆ Sakata R, Grant EJ, Ozasa K: Long-term follow-up of atomic bomb survivors. *Maturitas* 2012 (June); 72(2):99–103.

(RR 2-12) (related to *Life Span Study* and *Adult Health Study*) (refer to abstract in *Life Span Study* Publications)

◆ Samartzis D, Nishi N, Cologne JB, Funamoto S, Hayashi M, Kodama K, Miles EF, Suyama A, Soda M, Kasagi F: Ionizing radiation exposure and the development of soft-tissue sarcomas in atomic-bomb survivors. *J Bone Joint Surg Am* 2013 (February); 95(3):222–9. (RR 1-11) (refer to abstract in *Life Span Study* Publications)

Other Journal Publications

◆ Kodama K: Radiation health effects: radiation and cancer risk in atomic bomb survivors. *Medical Science Digest* 2012 (November); 38(13):20–3. (Japanese) (related to *Adult Health Study*)

◆ Kodama K, Ozasa K, Katayama H, Shore RE, Okubo T: Radiation effects on cancer risks in the Life Span Study cohort. *Radiat Prot Dosimetry* 2012 (October); 151(4):674–6. (related to *Life Span Study* and *Special Cancer Studies*)

◆ Sugiyama H, Ozasa K, Tanaka J, Kakehashi M, Tsunematsu M, Takeda N, Arita K, Kamada N: Childhood cancer incidence and circumstances regarding diagnosis and treatment in Hiroshima prefecture, 2004–2008. *Hiroshima Igaku [J Hiroshima Med Assoc]* 2012 (November); 65(11):685–95. (Japanese)

Tumor and Tissue Registries Oral Presentations

❖ Kodama K, Ozasa K. Epidemiological project on A-bomb survivors. 52nd Annual Meeting of the Japanese Respiratory Society, 20–22 April 2012, Kobe (related to *Life Span Study*)

❖ Nagayoshi A, Yoshida M, Inada Y, Hayama S, Yamakawa S, Yamada T, Soda M. Ascertainment of vital status by acquisition of residence certificates. 21st Annual Meeting on Japanese Association of Cancer Registries, 7–8 June 2012, Kochi

❖ Soda M, Iwanaga M, Soda H. Survival of lung cancer patients in Nagasaki Japan: An analysis of population-based cancer registry. 34th Annual Meeting of the International Association of Cancer Registries, 17–19 September 2012, Cork, Ireland

❖ Sugiyama H, Ozasa K, Tanaka J, Kakehashi M, Tsunematsu M, Takeda N, Arita K, Kamada N. Childhood cancer incidence and circumstances regarding diagnosis and treatment in Hiroshima Prefecture, 2004–2008. 34th Annual Meeting of the International Association of Cancer Registries, 17–19 September 2012, Cork, Ireland

❖ Sugiyama H. Cancer registry and its utilization. Prefectural Forum “Facing Cancer,” 2 March 2013, Hiroshima

Research Protocols 3-04, 1-92, 10-86, 18-59, A5-11, 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, Kodama Y (G), Cullings HM (S), Akahoshi M (CN), Tomonaga M, Iijima Y, Mine M

Objectives: To investigate the possibility that Nagasaki survivors who were exposed in factories have overestimated doses.

Background and Significance: It is difficult to estimate doses for Nagasaki survivors who were exposed in factories because of possible partial shielding by nearby machinery. Biodosimetric estimates can be used to validate or adjust the physical dose estimates. Tooth enamel from Nagasaki survivors, especially factory workers when available, will be measured by electron spin resonance (ESR) to evaluate radiation dose. Chromosome aberration frequency will be measured for the same donors. Based on the results, we will examine the validity of the factory worker DS02 dose estimates.

Study Methods: We use extracted teeth to measure the absorbed gamma-ray dose by ESR (^{60}Co gamma-ray equivalent dose). Measurements of chromosome aberration frequencies in blood lymphocytes will also be made from the same donors to compare the results.

Study Progress: We obtained 15 tooth samples in this fiscal year. All of them are seriously decayed.

Results and Conclusions: Tooth collection is slow. Thus far, 29 tooth samples were measured with ESR. Because the number of tooth samples examined was limited, it was not possible to derive a conclusion about a possible dose bias. However, a close correlation was found between the ESR dose estimate and the cytogenetic dose estimate on the same survivors. Thus, these results may encourage the use of cytogenetic data, which is much more abundant than tooth data, for a better understanding of uncertainties associated with individual DS02 doses.

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, Kodama Y (G), Wada T, Cullings HM (S), Ohishi W (CH), Rühm W, Wallner A, Wieser A

Objectives: To estimate the individual doses using teeth by the electron spin resonance (ESR) technique and to compare the results with DS02 dose, and with chromosome aberration frequencies in lymphocytes, from the same donors.

Background and Significance: This study plan is to measure CO_2^- radicals induced by gamma-ray exposure in tooth enamel by means of the ESR technique (Ikeya et al., *Jpn J Appl Phy* 1984; 23:L697). For this purpose, we collected teeth according to RP 10-86. Since the ESR signal intensity is linearly related to gamma-ray dose, this technique enables us to estimate the gamma-ray dose of the survivors. ESR has proved to be a good choice in that the ESR-estimated doses agreed well with the cytogenetically estimated doses and jointly provide a good comparison basis to evaluate the

physical dose estimates (DS02).

Study Methods: We use tooth enamel to measure the absorbed gamma-ray dose by ESR (^{60}Co gamma-ray equivalent dose). Measurements of chromosome aberration frequencies in blood lymphocytes are made from the same donors and the results are compared.

Study Progress: We measured 25 molars in this fiscal year. Thus far, a total of 274 molars donated by 214 Hiroshima AHS participants were measured.

Results and Conclusions: None yet.

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, Ohishi W (CH), Akahoshi M (CN)

Objectives: To collect teeth from Adult Health Study (AHS) subjects who were exposed to the bomb within 2 km from the hypocenter and controls (estimated dose <5 mGy).

Background and Significance: Electron spin resonance (ESR; also called electron paramagnetic resonance, EPR) was first used to measure the cumulative radiation dose to the enamel of teeth from A-bomb survivors by Okajima's group at Nagasaki University. As the ESR signal intensity is linearly related to gamma-ray dose, this technique enables us to estimate individual doses. The collection of teeth from A-bomb survivors is the first part of the project to estimate the radiation dose of A-bomb survivors.

Study Methods: The Department of Clinical Studies sends letters to AHS participants twice a year for health monitoring. A sentence in the letters expresses our continued interest in the collection of teeth.

Study Progress: A total of 36 teeth were received from Hiroshima AHS participants and 15 from Nagasaki AHS participants during FY2012.

Results and Conclusions: Thus far, we have collected 1,586 tooth samples from Hiroshima AHS participants during the past 25 years and 73 from Nagasaki AHS participants during the past eight years. On average, nearly 15% of the collected samples are suitable for ESR measurement (i.e., molars that are not seriously decayed).

RP 18-59 Shielding survey and dosimetry study

Cullings HM (S), Grant EJ (EH), Watanabe T (EH), Funamoto S (S), Matsumoto N (S), Cologne JB (S)

Objectives: The purpose of this protocol is to refine dose estimates for Hiroshima and Nagasaki atomic-bomb survivors and to characterize the uncertainties in these estimates.

Background and Significance: Accurate and detailed dose estimates are essential to characterizing the radiation dose response of any health effect under study. Three successive systems for calculating survivor doses based on location and shielding have been developed and implemented: T65D, DS86, and DS02.

Study Methods: DS02 is based on calculations of the nuclear explosions and the generation, transport, and alteration by shielding of the neutrons and gamma rays that directly irradiated survivors. Methods have been developed at RERF to improve input data on survivor locations and shielding, to extend the systems to longer distances and less

detailed shielding data than those for which the core system performs calculations, and to characterize uncertainty in individual dose estimates and reduce the impact of uncertainties on radiation risk estimates.

Study Progress: Pre-2012 – During 2007 a new committee was initiated by the Chairman, Dr. Okubo, to examine a number of issues (residual radiation, improvement of input data, factory worker doses, etc.). Four new collaborations on dose error and biodosimetry were begun with groups of external investigators. Dr. Okubo and others in the Dosimetry Committee devised methods to make new estimates of map coordinates for individual survivors using archival RERF data with current technology.

2012 – The Epidemiology and Statistics Departments have made progress in a number of areas:

- The Master File Section completed the process of aligning neighborhood diagrams from survivors' shielding histories with the aerial photographic mosaics for the LSS. They also completed checking of original source documents, e.g., Master Sample Questionnaires, for the most accurate U.S. Army map coordinates for all LSS members without shielding histories, including the restoration of ten-yard digits to coordinates that had been truncated in earlier years, and used a map coordinate conversion system to remove local distortions in the U.S. Army maps and convert coordinates to the new Japanese system JGD2000. This provides more accurate coordinates for all members of the LSS, in a contemporary geographical coordinate system. Statistics evaluated the effect of resulting changes in distance on survivor doses. We initiated a program to use terrain elevation data from purchased digital maps, with the new coordinates, for more complete and consistent calculation of terrain shielding for all members of the LSS, rather than limited subsets based on particular shielding categories.
- We continued collaborative research with several external researchers to develop and compare statistical methods to estimate dose uncertainties and minimize their effects on risk estimation, including use of biodosimetry data. Two manuscripts have been submitted by a collaborator team.
- We continued work on residual radiation, and Dr. Cullings presented at and co-chaired a DOE-sponsored session about that at the annual meeting of the Health Physics Society.

Results and Conclusions: Work in progress confirms the suggestion of DS86 that the population dose increment in person-gray from known sources of residual radioactivity is a small fraction of direct doses calculated by DS02. Preliminary geospatial analyses of various biological endpoints have not shown any patterns consistent with undocumented doses from residual radiation or other deficiencies in the dosimetry. Work on other aspects mentioned above is ongoing.

RP-A5-11 Bio-dosimeters as instrumental variables for assessing and dealing with uncertainty in atomic-bomb survivor dose estimates: Preliminary evaluation in the AHS sub-cohort

Carter R, Miller A, Tekwe C, Cullings HM (S), Neriishi K, Kodama Y (G), Kusunoki Y (R), Nakamura N, Ozasa K

(EH), Imaizumi M (CN), Cologne JB (S), Nakashima E (S), Misumi M (S), Funamoto S (S), Stram DO, Douple EB (ACR)

Objectives: To develop and evaluate methods for dealing with dosimetry error in radiation risk estimation using ancillary information contained in various bio-dosimeters and biomarkers measured in a subset of the AHS cohort. The goal will include publications in substantive journals in addition to statistical journals.

Background and Significance: Inaccuracies and unknown quantities in the estimation of atomic-bomb survivor radiation doses can lead to bias and greater uncertainty in risk estimation. To date, methods for dealing with dosimetry errors have focused on substitution of expected doses based on an assumed model for the dose measurement-error distribution including assumed parameter values for the variance. If the assumption is incorrect, then the method leads to biased estimates of dose-response relationships. Incorporating information on bio-dosimetric measurements could be used to address this issue.

Study Methods: The intent of this work is to develop approaches that incorporate information from biodosimeters or biomarkers to estimate measurement error variances. A method that treats biodosimetric data as so-called “instrumental variables” (i.e., variables that are correlated with true dose but uncorrelated with measurement error) can obviate the need to assume a known error-variance parameter. The data will consist of DS02 measurements and potential bio-dosimetric data from chromosome aberration, clinical/laboratory measurements and disease incidence.

Study Progress: Two graduate students, Austin Miller and Carmen Tekwe, have completed Ph.D. dissertations on relevant methods. Tekwe produced two manuscripts related to her dissertation, which have been submitted to journals.

Results and Conclusions: Analyses by the two graduate students have produced estimates that were reasonably consistent with the assumed values of error variance currently used in the methods employed by RERF for calculating “adjusted” survivor doses.

Methods based on instrumental variables are useful in obtaining estimates of the size of overall measurement error in RERF dosimetry (e.g., DS02) data, subject to the assumptions related to the parametric form of the error model for the instrumental variable(s) analysis, i.e., the probability distribution of errors in the survivor’s calculated DS02 doses.

RP-A4-10 Semiparametric 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, Kodama Y (G), Davis S, Kopecky KJ

Objectives: Use semiparametric statistical methods to adjust for the effects of radiation dose measurement error on the estimation of radiation dose responses, incorporating information from biodosimetric data available on a part of the cohort.

Background and Significance: Although some measurement error methods have been applied to adjust for radiation measurement error in RERF data, further develop-

ment of semiparametric or nonparametric methods 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.

Study Methods: The AHS subcohort of about 4,000 who have DS02 radiation dose estimates, plus stable chromosome aberration data, and outcome data for diseases such as cardiovascular heart disease, stomach cancer, lung cancer, or breast cancer, will comprise the calibration sample. By using data from the calibration sample, we can estimate radiation dose responses for the entire LSS, with an appropriate adjustment for the uncertainty in DS02 dose estimates. An important result here is that the measurement error standard deviation will not have an assumed value, but rather will be estimated from the data using an innovative method, even though the data do not include replicate measurements/estimates of radiation doses. We will consider logistic, Cox, and additive hazard regression models for the radiation dose response, with adjustment for smoking information, age at exposure, gender, education, and city.

Study Progress: A U.S. National Institute of Health (NIH) grant was obtained (CY Wang, PI) to support the work. Statistical analysis is in progress. One related paper has been published and two others have been submitted by the non-RERF collaborators; these develop the method but do not use RERF data.

Results and Conclusions: Several methods have been developed that utilize different methods and different assumptions, for different situations in terms of available surrogate and instrumental variables and types of regression analysis.

Atomic-bomb Dosimetry Studies Oral Presentations

❖ Pierce DA, Cullings HM, Kellerer A. Accounting for neutron doses received by the Japanese atomic-bomb survivors. 2012 ASA (American Statistical Association) Conference on Radiation and Health, 10–13 June 2012, Kennebunkport, Maine, USA

❖ Cullings HM. Doses received by atomic-bomb survivors in the Life Span Study Cohort from known residual radiation sources in Hiroshima and Nagasaki. 57th Annual Meeting of the Health Physics Society, 22–26 July 2012, Sacramento, California, USA

❖ Cullings HM. The development of the dosimetry for the Japanese atomic-bomb survivors and its implications for other radiation studies. 9th International US-Russian JCCRER Meeting, 22–25 October 2012, San Francisco, California, USA